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INDIAN AGRICULTURAL
RESEARCH INSTITUTE, NEW DELHI.

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UNION OF SOUTH AFRICA

DEPARTMENT OF AGRICULTURE

THE
ONDERSTEPOORT JOURNAL
OF
VETERINARY SCIENCE
AND
ANIMAL INDUSTRY

VOL. I JULY, 1933 No. 1

PUBLISHED QUARTERLY

Edited by: P. J. DU TOIT, Director.

The Government Printer, Pretoria, South Africa
1933

577-23 5/33-1,400

DEPARTMENT OF AGRICULTURE,
DIRECTOR OF VETERINARY SERVICES AND ANIMAL INDUSTRY,
ONDERSTEPOORT LABORATORIES,
PRETORIA, SOUTH AFRICA,
July, 1933.

List of Reports issued by the Director of the Onderstepoort Laboratories.

Report of the Government Veterinary Bacteriologist of the Transvaal for the year 1903-4.*

Report of the Government Veterinary Bacteriologist of the Transvaal for the year 1904-5.*

Report of the Government Veterinary Bacteriologist of the Transvaal for the year 1905-6.*

Report of the Government Veterinary Bacteriologist of the Transvaal for the year 1906-7.*

Report of the Government Veterinary Bacteriologist of the Transvaal for the year 1907-8.*

Report of the Government Veterinary Bacteriologist of the Transvaal for the year 1908-9.*

Report of the Government Veterinary Bacteriologist of the Transvaal for the year 1909-10.*

First Report of the Director of Veterinary Research, August, 1911.*

Second Report of the Director of Veterinary Research, October, 1912.*

Third and Fourth Reports of the Director of Veterinary Research, November, 1915.*

Fifth and Sixth Reports of the Director of Veterinary Research, April, 1918.*

Seventh and Eighth Reports of the Director of Veterinary Research, April, 1918.*

Ninth and Tenth Reports of the Director of Veterinary Education and Research, April, 1923.

Eleventh and Twelfth Reports of the Director of Veterinary Education and Research Part I, September, 1926.

Eleventh and Twelfth Reports, of the Director of Veterinary Education and Research, Part II, January, 1927.

Thirteenth and Fourteenth Reports of the Director of Veterinary Education and Research, Parts I and II, October, 1928.

Fifteenth Report of the Director of Veterinary Services, Parts I and II, October, 1929.

Sixteenth Report of the Director of Veterinary Services and Animal Industry, August, 1930.

Seventeenth Report of the Director of Veterinary Services and Animal Industry, Parts I and II, August, 1931.

Eighteenth Report of the Director of Veterinary Services and Animal Industry, Parts I and II, August, 1932.

Onderstepoort Journal of Veterinary Science and Animal Industry, Vol. I, No. 1, July, 1933.

P. J. DU TOIT,
Director of Veterinary Services and Animal Industry.

*Now out of print.

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FOREWORD.

SINCE the foundation of the Onderstepoort Veterinary Research Station twenty-five years ago, the results of research work carried out at this Institution have been published in the form of "Annual Reports." This form of publication will, in future, be departed from only as regards title and frequency of publication. The intention is that *The Onderstepoort Journal of Veterinary Science and Animal Industry* should form a direct continuation of the "*Reports of the Director of Veterinary Services and Animal Industry*," and be published quarterly instead of annually. Two quarterly numbers will form one volume, so that two volumes should be completed every year.

The advantages of publishing at short intervals are obvious. Nothing is more disheartening to the research worker than to see the publication of his results delayed for months or years. It is also hoped that the change in the title of the publication will prove to be of value. "Reports" are apt to be regarded as a mere summary of routine duties or a brief indication of the work performed during the year. Such a report which incorporates a summary of the activities of the Division of Veterinary Services and Animal Industry is actually published annually by the Department of Agriculture in *Farming in South Africa*. But the "*Reports of the Director of Veterinary Services and Animal Industry*" were strictly scientific volumes containing original articles written by the research workers themselves. It is felt that the term "*Journal*" will indicate the nature of this publication better than the term "Report."

Finally, the hope is expressed that the new Journal will find a worthy place on the library shelves of all Institutes interested in biological problems.

Onderstepoort,
July, 1933.

P. J. DU TOIT.

Section I.

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Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 1, Number 1, 1933.

Mortality in Fowls due to *Aegyptianella pullorum*.

By J. D. W. A. COLES, B.V.Sc., Veterinary Research Officer, Onderstepoort.

THE purpose of this article is to record three outbreaks of Aegyptianellosis accompanied by mortality. Although the literature contains several references to the parasite, it is still uncertain to what extent it may cause death. Each outbreak will be described separately.

The first outbreak was reported on 24.8.32 at Pretoria North, near Onderstepoort. The poultryman on 10.8.32 had bought two hundred day-old chicks and placed them under a cold brooder in a small corrugated iron shed. On 23.8.32 he found three dead chicks, and others which were slightly weak, not eating and showing a marked bright green diarrhoea. On 24.8.32 five died and two were sent for examination. At autopsy each showed marked anaemia, intense icterus, atony of the crop, intestinal catarrh (the intestines were full of green slimy material), yellowish and slightly enlarged liver, and marked tumor splenis; the kidneys had a very pronounced yellowish green colour, and the blood and spleen smears showed anaemic changes and numerous *A. pullorum*.

On 25.8.32 there were twelve chicks dead. The lesions and blood smears resembled those of the day before, but one blood smear showed *Spirochaeta anserina* as well as *A. pullorum*. This day the author visited the farm and found the brooder and shed fairly badly infested with *Argas persicus* (Oken). Several of the chicks were ailing, not eating and manifesting markedly greenish diarrhoea. The owner was advised to destroy all *A. persicus*, and this he did immediately. On 26.8.32 nine more chicks were dead. All smears showed *A. pullorum*. On 27.8.32 six died; on 28.8.32 fourteen; on 29.8.32 fifteen; on 30.8.32 nineteen; on 31.8.32 again nineteen; on 1.9.32 five; on 2.9.32 five, and on 3.9.32 one died. No further cases occurred. During this time a number of chicks were examined. All showed the post-mortem lesions described, together with *A. pullorum*. Apart from *A. persicus*, no other ectoparasites were ever found. Argas larvae were never found on the chicks. Occasionally live sick chickens were examined and the blood showed *A. pullorum*, but never *S. anserina*. In this outbreak one hundred and thirteen chickens out of two hundred died within the first twenty-five days of life and within twenty-four days of being exposed to *A. persicus*. The first deaths occurred thirteen days after exposure to *A. persicus*. The mortality stopped nine days after the eradication of the ticks; this fact has some significance because mortality might reasonably have been expected for at least another four days (*vide* article in this Report by Bedford and Coles). This discrepancy, too, would seem to support an idea founded purely on circumstantial evidence that the fowl is most susceptible to Aegyptionellosis when infected during the first week of life. All the chickens showed the same symptoms and, except in the one case where spirochaetes also were seen, every chick examined showed a pure infection of *A. pullorum*. If *A. pullorum* represented a stage in the life cycle of *S. anserina*, it is extremely difficult to understand why spirochaetes were encountered only once, specially as the incubation period of *S. anserina* is approximately only half that of *A. pullorum*, and several chicks were examined immediately symptoms were noted and presumably before a crisis could have occurred. Moreover, as will be seen in the article of Bedford and Coles referred to, clean *A. persicus* adults were fed on some of these chickens and subsequently transmitted only *A. pullorum* to healthy clean chickens. It seems safe to assert that *A. pullorum* was responsible for the heavy mortality in these chickens.

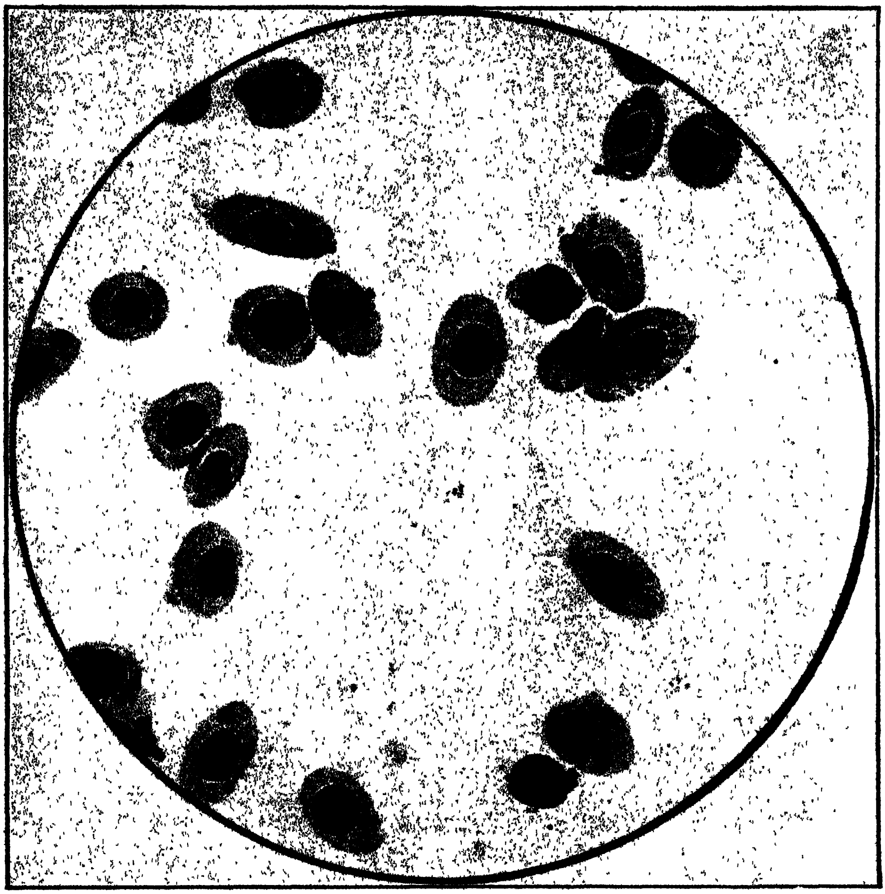


Fig. 1. 1000×. Infected red blood corpuscles. The very small specks in the cells are the "merozoites" liberated by the breaking down of schizonts.

The second outbreak occurred at the beginning of November, 1932, in Pretoria. A poultryman brought one dead chick, and one live one about eight weeks old, for examination as he had been experiencing heavy mortality. The dead chick was very anaemic, had an enlarged spleen and an enlarged greenish brown and mottled liver, and the blood smear showed anaemia and a few *A. pullorum*.

The live chick showed a marked orange yellow comb, and the iris had a jaundiced appearance; the blood smear showed marked anaemia and fairly numerous *A. pullorum*. This chick died shortly after arrival and showed icterus, tumor splenis, anaemia and slightly yellowish liver, slightly enlarged and pale kidneys and intestinal catarrh. Two days later the owner brought twenty-six supposedly sick chickens for examination. Of these, five showed *A. pullorum* and seven showed *S. anserina* in blood smears. During the next two days six of these chickens died, all from Spirochaetosis. The others all recovered. The owner admitted his premises were not free of ***A. persicus***. Undoubtedly most of the deaths in these chickens one to two months old were due to Spirochaetosis. It is possible that the two supposed to have died from Aegyptianellosis had really succumbed to Spirochaetosis, the spirochaetes having already disappeared.

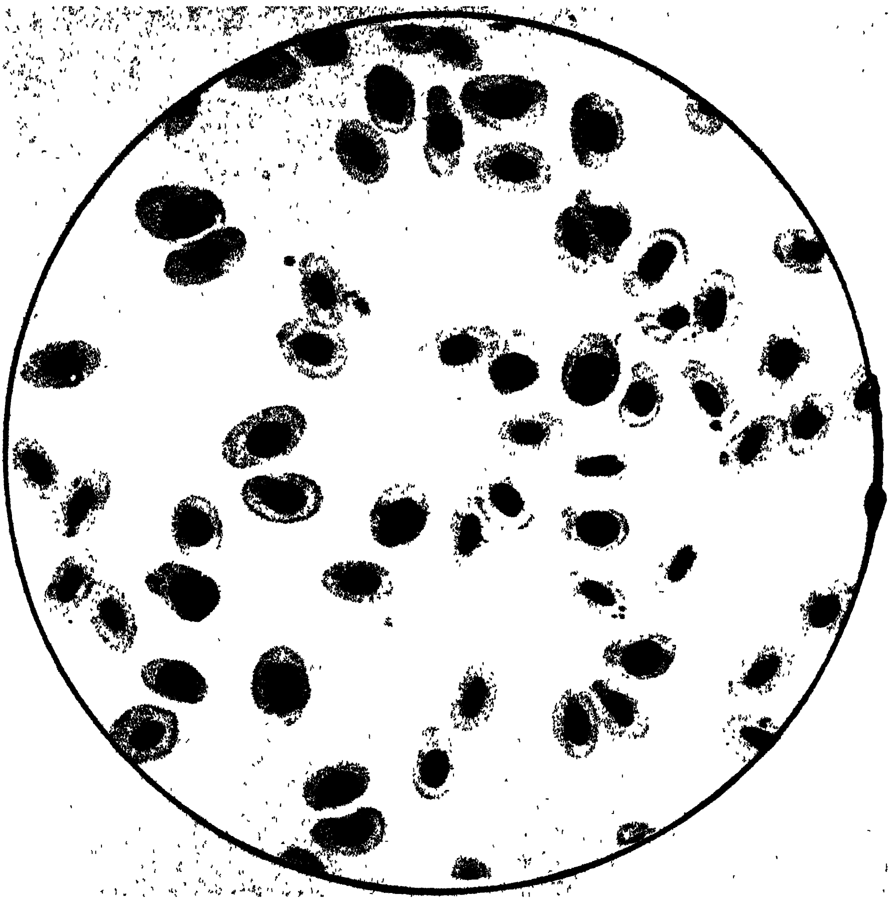


Fig. 2. 1000×. Cells showing fairly numerous "merozoites", the arrangement of which round the periphery is very characteristic. Some blood smears show practically nothing but these "merozoites".

The third outbreak occurred at Pietersburg in the Northern Transvaal. On 6.12.32 a farmer sent a live Rhode Island Red cockerel about five months old for examination. The bird had been ill for a few days. On arrival it had a temperature of 108° F.; it was listless, slightly emaciated, had a pale yellowish comb and fairly marked greenish diarrhaea; the blood smear showed *A. pullorum* numerous. Four days later the bird died and at autopsy was noted a fairly marked tumor splenis, anaemia, slight icterus and slight enlargement of the liver; the intestines contained greenish slimy material. The owner said only three out of two hundred birds on free range became affected and the other two died. Here again it may be argued that Spirochaetosis was really the cause of death, although no spirochaetes were found. The author prefers to regard the deaths as being due to Aegyptianellosis, as it is most unusual to find only two or three isolated deaths due to *Spirochaetosis*. A pure strain of *A. pullorum* was obtained by feeding clean adults of *A. persicus* on this fowl.

SUMMARY AND CONCLUSIONS.

Three outbreaks of *Aegyptianellosis* have been described, in the first of which there can be no reasonable doubt that the disease caused very heavy mortality in very young chicks. In the other outbreaks there is evidence to show that *A. pullorum* may kill older fowls, but apparently not to the same extent as *S. anserina*. It is interesting to note that under laboratory conditions the disease transmitted by infected *A. persicus* to chickens three to eight weeks old apparently does very little harm. The factors affecting the virulence of *A. pullorum* are still obscure, but the age of the bird appears to be of some importance. The main symptoms in chickens are loss of appetite, dejection and diarrhoea. Sometimes icterus may be noted. At autopsy very young chicks show intense icterus and anaemia,

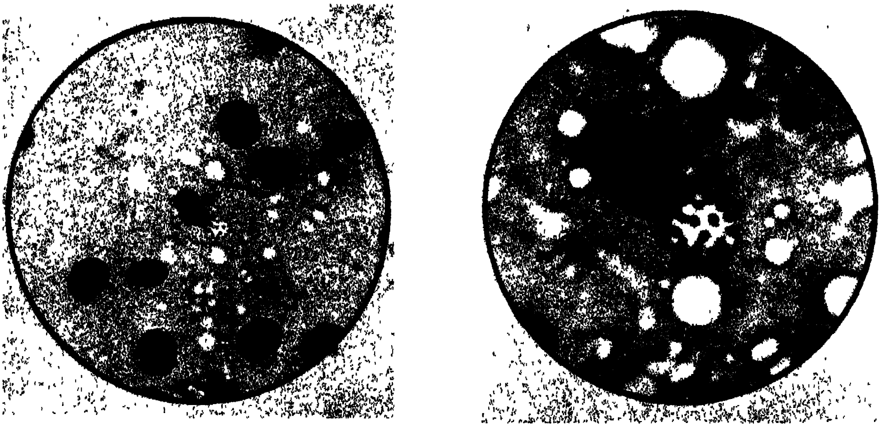
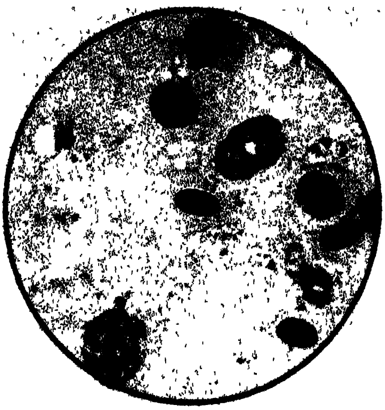


Fig. 3. 1000×. Red corpuscle containing a schizont.
Fig. 4. 2700×. No. 3 magnified.

marked tumor splenis, fatty degeneration of the liver, intestinal catarrh, and a characteristic pronounced pale greenish yellow colour of the kidneys. The risk of mortality seems to decrease with age, and then the symptoms and gross post-mortem findings are apparently indistinguishable from those of spirochaetosis.



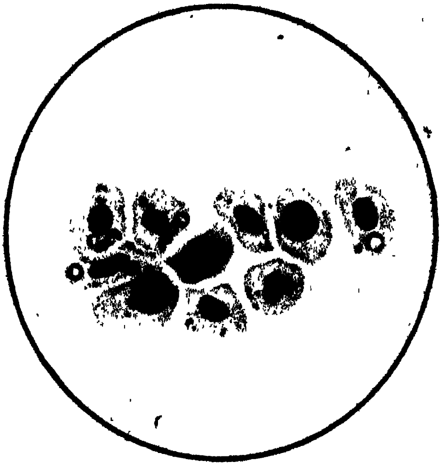


Fig. 5. 1000×. Red cell packed with " merozoites " formed by the breaking up of a schizont.

Fig. 6. 1000×. Infected red cells. The numerous " merozoites " are very noticeable.

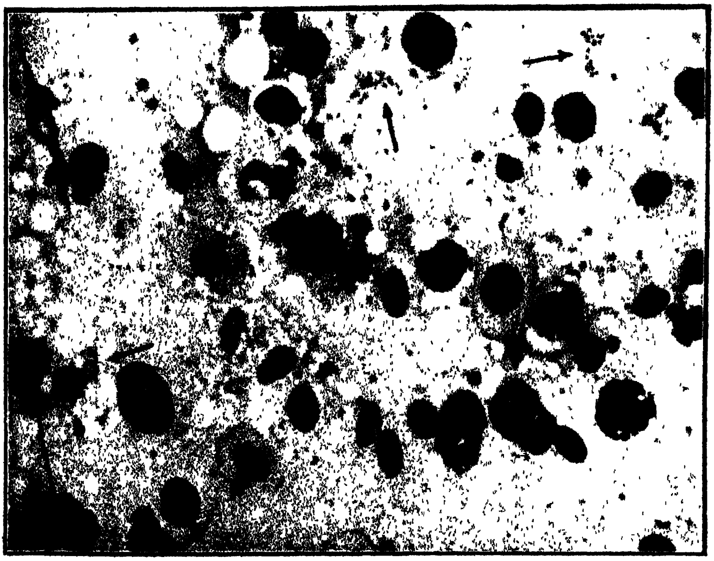


Fig. 7. 1000×. This is a spleen smear. The arrows indicate masses of " merozoites ". A cell containing a schizont is shown.

ACKNOWLEDGMENT.

The author wishes to thank Mr. T. Meyer for the preparation of the photo-micrographs, which give an excellent idea of the different forms in which *A. pullorum* manifests itself in the blood of fowls.

REFERENCE.

BEDFORD, G. A. H., AND COLES, J. D. W. A. (1933). The Transmission of *Aegyptianella pullorum*, Carpano. to Fowls by means of Ticks belonging to the Genus *Argas*. This Journal.

Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 1, Number 1, 1933.

The Transmission of *Aegyptianella pullorum*, Carpano, to Fowls by means of Ticks belonging to the Genus *Argas*.

By G. A. H. BEDFORD, Research Officer, Onderstepoort; and
J. D. W. A. COLES, B.V.Sc., Veterinary Research Officer, Onderstepoort.

EXPERIMENTS were undertaken in order to ascertain whether *Argas persicus* (Oken), *A. moubata* Murray and *A. perengueyi* (Bedford and Hewitt) were transmitting agents of *Aegyptianella pullorum* to fowls.

TECHNIQUE.

The ticks used for the tests were kept in the laboratory at room temperature, the maximum temperature during the day varying between about 24 and 29°C, and the minimum temperature at night varying between about 20 and 23·3°C. They were fed by liberating them along with chickens in an open glass box measuring 25·5 × 38 cm. and 35·5 cm. high. In the bottom of the box was placed a sheet of brown paper and on top of this a wooden grid. The ticks were placed on the paper, and the grid prevented the chickens from getting at the ticks and eating them. The ticks and chickens were placed in the box in the evenings, and taken out again on the following mornings. Only in the case of the adults of *A. perengueyi*, which refused to feed on the healthy chickens, was it found necessary to tie each bird down on a table and keep the tick on its skin during the night by placing a small square piece of bandage over the tick and sticking the edges of the bandage to the skin with collodion. The bandage was removed the following morning by damping and rubbing the edges with cotton wool soaked in ether.

Birds infected with the larvae of *A. persicus* were kept in small cages, and a tray containing water, into which the ticks fell after feeding on their hosts, was placed beneath each.

The first batches of larvae that were used were placed on chickens that were only a few days old, and as they could never be found on the birds afterwards, it would appear that very young chickens are more sensitive to the bites of the ticks than older birds, and being less covered with feathers, are more easily able to find the ticks and eat them. When older chickens were used the larvae were found on them without difficulty.

Two strains of *A. pullorum* were used, one being obtained from a poultry keeper at Pretoria North, who had sustained severe losses amongst his young chickens, and the other from Pietersburg.

To ensure that all the healthy birds used for these experiments had never been infected with *A. pullorum*, only young chickens kept, after hatching, in tick-free places were used.

EXPERIMENTS WITH *Argas persicus* (OKEN).

Experiment 1.

1.ix.32.—1 adult *A. persicus* fed on infected chicken (A). Pretoria North strain.

6.x.32—Fed on healthy chicken (No. 1). Feeding interval 35 days.

10.ii.33.—Fed on healthy chicken (No. 7), five months after feeding on infected chicken.

Results.—Chicken No. 1 showed parasites, very rare, in the blood on 19.x.32. On the 21st parasites were frequent and there were marked anaemic changes. Died as a result of *A. pullorum* and secondary bacteraemia on the 22nd. Incubation period 13 days.

Chicken No. 7 showed parasites in the blood on 25.ii.33, and also on subsequent occasions, proving that an infected tick can infect more than one chicken. Incubation period 15 days.

Experiment 2.

1.ix.32.—2 adults *A. persicus* fed on infected chicken (A). Pretoria North strain.

15.x.32.—Fed on 2 healthy chickens (Nos. 2–3). Interval between feeding 44 days.

27.i.33.—Adult No. 1 fed on healthy chicken (No. 8).

Adult No. 2 refused three times to feed on healthy chicken (No. 9).

Results.—Chicken No. 2 died through accident on 20.x.32. No parasites found in the blood.

Chicken No. 3 showed parasites in the blood on 27.x.32, and also on subsequent occasions. Incubation period 12 days.

Chicken No. 8 showed parasites in the blood on 9.ii.33. and also on subsequent occasions. Incubation period 13 days.

Experiment No. 3.

3.ix.32.—4 *A. persicus* (3 adults, 1 nymph) fed on infected chicken (B). Pretoria North strain.

18.x.32.—Fed on 2 healthy chickens (Nos. 4–5). Interval between feeding 45 days.

Results.—Chickens Nos. 4 and 5 showed parasites, very rare, in the blood on 31.x.32. Incubation periods 13 days.

Experiment No. 4.

21.x.32.—2 adults *A. persicus* fed on infected chicken (C). Pretoria North strain.

1.ii.33.—1 adult fed on healthy chicken (No. 11). Interval between feeding 102 days.

Results.—The result was positive, parasites being found infrequent on 3.iii.33. It is not known, however, when the parasites first appeared in the blood of the chicken.

Experiment No. 5.

7.xii.32.—1 adult *A. persicus* fed on infected fowl (E). Pietersburg strain.

3.ii.33.—Fed on healthy chicken (No. 13). Interval between feeding 26 days.

Result.—Chicken showed parasites in the blood on 15.ii.33, and also on subsequent dates. Incubation period, 12 days.

Experiment No. 6.

7.xii.32.—1 adult *A. persicus* fed on infected fowl (E). Pietersburg strain.
25.ii.33.—Fed on healthy chicken (No. 14). Interval between feeding 79 days.

Result.—Chicken showed parasites in the blood on 11.iii.33. Incubation period 14 days.

EXPERIMENTS WITH *Argas moubata*, MURRAY.

Experiment No. 7.

30-31.x.32.—1 nymph (♂) *A. moubata* fed on infected chicken (D). Pretoria North strain. Parasites were rare in the blood on the 31st, only one being found per field.
25.xi.32.—Fed as adult on healthy chicken (No. 6). Interval between feeding 24-25 days.

Result.—Chicken showed no signs of infection.

Experiment No. 8.

30-31.x.32.—1 female *A. moubata* fed on infected chicken (D). Pretoria North strain.
2.ii.33.—Tick gorged on healthy chicken (No. 12). Interval between feeding 93 94 days.

Result.—Chicken showed no signs of infection.

Experiment No. 9.

30–31.x.32.—1 male *A. moubata* fed on infected chicken (D). Pretoria North strain.
24.ii.33.—Fed on healthy chicken (No. 16). Interval between feeding 115–116 days.

Result.—Chicken showed no signs of infection.

Experiment No. 10.

30–31.x.32.—1 male *A. moubata* fed on infected chicken (D). Pretoria North strain.
28.ii.33.—Fed on healthy chicken (No. 17). Interval between feeding 119–120 days.

Result.—Chicken showed no signs of infection.

Experiment No. 11.

8.ii.33.—10 nymphs, whose mother fed on infected chicken (D) on the 30–31.x.32, fed on healthy chicken (No. 15).

Result.—Chicken showed no signs of infection.

EXPERIMENTS WITH *Argas perengueyi* (BEDFORD & HEWITT).

Experiment No. 12.

1.xi.32.—1 female *A. perengueyi* fed on infected chicken (D). Pretoria North strain. Parasites frequent in blood.
3.iii.33.—Tick gorged on healthy chicken (No. 10). Interval between feeding 4 months.

Result.—Chicken showed no signs of infection.

Experiment No. 13.

1.xi.32.—1 male *A. perengueyi* fed on infected chicken (D). Pretoria North strain. Parasites frequent in blood.

4.iii.33.—Tick gorged on healthy chicken (No. 18). Interval between feeding 4 months.

Result.—Chicken showed no signs of infection.

Experiment No. 14.

30–31.x.32.—1 male *A. perengueyi* fed on infected chicken (D).

7.iii.33.—Placed on healthy chicken (No. 19).

The tick could not be found the following morning, and it is therefore not known whether it fed or not.

Result.—Chicken showed no signs of infection.

SUMMARY AND CONCLUSIONS.

A. persicus is a transmitting agent of *Aegyptianella pullorum* to fowls. The disease was successfully transmitted to 9 healthy chickens by adult ticks which had previously fed on infected chickens. Moreover, the tick can retain its infection after feeding on a healthy bird, as one adult transmitted the disease twice to healthy chickens. The shortest interval between feeding on an infected bird and a healthy chicken was 26 days. The incubation period in chickens after an infected tick has bitten varies from 12 to 15 days or more. An infected adult *A. persicus* may remain infected for at least 162 days. *A. pullorum* is apparently not transmitted by either *Argas moubata* or *A. perengueyi*, as four adults of *A. moubata* and two or three adults of *A. perengueyi* failed to transmit the disease to healthy chickens after having fed on infected birds. Nymphs of *A. moubata*, whose mother had fed on an infected chicken likewise failed to transmit the disease to a healthy chicken.

It is noteworthy that *Spirochaeta anserina* was never seen in any fowl in these experiments.

Section II.

Virus Diseases.

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P. J. DU TOIT, R. A. ALEXANDER, and W. O. NEITZ	The Immunization of Horses against Horsesickness by the use of formalised Virus, Part II	25
W. O. NEITZ and A. D. THOMAS	Rabies in South Africa. Occurrence and Distribution of Cases during 1932	51

Onderstepoort Journal of Veterinary Science and Animal Industry, Volume I, Number 1, 1933.

The Immunization of Mules with Formalysed Horsesickness Virus. II.

P. J. DU TOIT, B.A., Dr.Phil., Dr.Med.Vet., D.Sc., Director of Veterinary Services and Animal Industry;

R. A. ALEXANDER, B.Sc. Agric., B.V.Sc.. Empire Marketing Board Research Fellow; and

W. O. NEITZ, B.V.Sc., Veterinary Research Officer, Onderstepoort.

INTRODUCTION.

In 1932 du Toit and Neitz reported upon the application of the formalysed spleen virus method to the immunization of mules. They showed that a concentration of one part of formaldehyde to 3,000 parts of a 20 per cent. spleen emulsion could not be injected into mules in a dose of 30 c.c. with safety. The majority of the mules which survive this single injection are solidly immune, but on receiving an intravenous injection of 5 c.c. of O virus as an immunity test a small proportion may develop horsesickness and die.

In a second experiment upon a total of 40 mules the single immunizing dose was replaced by two injections at an interval of 14 days, the first injection consisting of virus inactivated by one part of formaldehyde to either 2,000 or 2,500 parts of spleen emulsion. The second injection one part of formaldehyde to 3,000 parts of emulsion. As a result of the immunizing injections no deaths occurred, in fact no clinical symptoms developed, but on applying an immunity test by injecting O virus intravenously two animals developed typical horsesickness and died.

This mortality of only 5 per cent. was considered quite satisfactory, more especially when it is considered that the immunity test was exceedingly severe, in fact far more severe than would be encountered under field conditions. It was therefore decided to repeat the experiment, and at the same time to introduce our modification namely, for one group of mules, to emulsify the infective spleen material in Tyrode solution instead of saline. Also the second injection was made one part of formaldehyde to 3,500 parts of emulsion.

OBJECT OF THE EXPERIMENT (S. 4718 and 4719).

To verify the previous results obtained with the subcutaneous injection of formalysed spleen material and to ascertain whether Tyrode solution has any advantage over 0·85 per cent. saline, as a vehicle for the virus.

METHOD.

The technique of preparation of the vaccine did not differ from that employed in previous experiments (du Toit and Neitz, 1932). The Tyrode solution was made up according to the formula given by Fisher namely, $NaCl$ 3 gm., KCl 0·20 gm., $CaCl_2$ 0·20 gm., $MgCl_2$ 0·10 gm., NaH/PO_4 0·05 gm., $NaHCO_3$ 1·0 gm., Glucose 1 gm., H_2O add 1,000 c.c.

Sterilization was performed by filtration through a Seitz filter under positive pressure. Spleens from horses 20286 and 20313 destroyed *in extremis* on the 4th and 5th days following the intravenous injection of 5 c.c. of O virus were used for the preparation of the vaccine. Approximately half of each spleen was used for the saline and the Tyrode emulsions; before injection the two respective portions were mixed.

The vaccine was given subcutaneously in 30 c.c. doses at an interval of 21 days. Twenty-one days after the second injection 5 c.c. of O virus was given intravenously as an immunity test.

The majority of the mules were quite unbroken and all were too wild to permit the taking of daily temperatures. The reaction to any injection therefore could only be gauged by careful daily observations on the general habitus of the animals. The results are indicated in Tables I and II.

RESULTS.

(a) *Saline emulsion.*—Of eleven mules injected one developed typical Dunkop Horsesickness to which it succumbed on the 18th day, the reaction being due to the injection of the 1 : 2,000 formaldehyde material; one mule showed diarrhoea on the 14th day; the remainder showed no apparent departures from normal health. On applying the immunity test the ten survivors proved to be solidly immune, no clinical symptoms of Horsesickness being observed at any time.

(b) *Tyrode Emulsion.*—Of ten mules injected one developed typical Dunkop Horsesickness and died on the 9th day after the 1 : 2,000 injection: one developed a mild but typical Dikkop attack from the 15th day but made an uneventful recovery. The remainder showed no clinical symptoms. On immunity test the nine survivors were solidly immune to O virus.

CONCLUSIONS.

No significant difference could be ascertained in favour of either saline or Tyrode solution as a fluid for emulsifying infective spleens. In both groups one animal died, but no importance can be attached to the fact that in the Tyrode group an additional animal developed clinical Horsesickness since individual susceptibility must be considered an important factor amongst small groups of only 10 animals.

2. It is confirmed that spleen virus attenuated by a concentration of one part of formaldehyde to 2,000 parts of infective spleen emulsion cannot be injected subcutaneously into mules with safety. Probably a concentration of 1 : 1,500 would be adequate.

3. The 1 : 2,000 formaldehyde concentration produces an immunity sufficient to protect against the 1 : 3,500 concentration. Mules which survive the 1 : 3,500 injection are solidly immune to fully virulent O virus.

4. The degree of immunity set up by 1/3,500 formaldehyde concentration is greater than that set up by 1 : 3,000 formalysed material as will readily be seen by a comparison with the results in the previous report.

5. The general result in previous work is confirmed namely that formalization of infective spleen material is capable of attenuating the virus so that it will become relatively avirulent but antigenic. The method as developed so far is not free from danger as it can be expected that 5 per cent. of animals will develop Horsesickness and die.

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TABLE I (SALINE EMULSION).

Experiment (*S.* 4718).

	D.O.B. No. of Mules.	Concentration of Formaldehyde in spleen emulsion.		Interval in days between injections.	Result of Immunization.	Date of injection of O virus.	IMMUNITY TEST.	
		1/2,000 M.	1/3,500 M.				Interval in days since last injection.	Result.
1....	20397	22.6.32	13.7.32	21	No clinical symptoms noticed	3.8.32	21	No clinical symptoms observed. Immune to Horsesickness.
2....	20398	22.6.33	13.7.32	21	14 days after injection of 1/2,000 mule developed diarrhoea	3.8.32	21	,, ,, ,,
3....	20399	22.6.32	13.7.32	21	No clinical symptoms noticed	3.8.32	21	,, ,, ,,
4....	20400	22.6.32	—	—	Showed typical clinical Dunkop Horsesickness reaction and died on the 18th day after 1/2000 injection	—	—	—
5....	20401	22.6.32	13.7.32	21	No clinical symptoms noticed	3.8.32	21	No clinical symptoms observed. Immune to Horsesickness.
6....	20402	22.6.32	13.7.32	21	,, ,, ,,	3.8.32	21	,, ,, ,,
7....	20403	22.6.33	13.7.32	21	,, ,, ,,	3.8.32	21	,, ,, ,,
8....	20404	22.6.32	13.7.32	21	,, ,, ,,	3.8.32	21	,, ,, ,,
9....	20405	22.6.32	13.7.32	21	,, ,, ,,	3.8.32	21	,, ,, ,,
10....	20406	22.6.32	13.7.32	21	,, ,, ,,	3.8.32	21	,, ,, ,,
11....	17897	18.8.32	8.9.32	21	,, ,, ,,	6.10.32	28	,, ,, ,,

TABLE II (TYRODE EMULSION).

Experiment (*S.* 4719).

	D.O.B. No. of Mule.	Concentration of Formaldehyde in Spleen Emulsion.		Interval in days between injections.	Result of immunization.	Date of injection of O virus.	Immunity Test.	
		1·2,000 S.	1/3500 S.				Interval in days since last injection.	Result.
1....	20377	22.6.32	13.7.32	21	No clinical symptoms noticed	3.8.32	21	No clinical symptoms observed. Immune to Horsesickness.
2....	20378	22.6.32	13.7.32	21	,, ,, ,,	3.8.32	21	,, ,, ,,
3....	20379	22.6.32	13.7.32	21	,, ,, ,,	3.8.32	21	,, ,, ,,
4....	20380	22.6.32	13.7.32	21	,, ,, ,,	3.8.32	21	,, ,, ,,
5....	20381	22.6.32	13.2.33	21	15 days after the injection of 1 2.000 mule showed typical clinical symptoms of Horsesickness and recovered	3.8.32	21	,, ,, ,,
6....	20382	22.6.32	13.7.32	21	No clinical symptoms noticed	3.8.32	21	,, ,, ,,
7....	20383	22.6.32	13.7.32	21	,, ,, ,,	3.8.32	21	,, ,, ,,
8....	20384	22.6.32	—	—	Showed typical clinical Dunkop Horsesickness reaction and died on the ninth day after injection of 1 2.000	—	—	—
9....	20385	22.6.32	13.7.32	21	No clinical symptoms noticed	3.8.32	21	No clinical symptoms observed. Immune to Horsesickness.
10....	20386	22.6.32	13.7.32	21	,, ,, ,,	3.8.32	21	,, ,, ,,

Onderstepoort Journal of Veterinary Science and Animal Industry, Volume I, Number 1, 1933.

The Immunization of Horses against Horse-sickness by the use of Formalysed Virus—Part II.

By P. J. DU TOIT, B.A., Dr. Phil., Dr. Med. Vet., D.Sc., Director of Veterinary Services and Animal Industry.

R. A. ALEXANDER, B.Sc., Agric. B.V.Sc., Empire Marketing Board Research Fellow.

W. O. NEITZ, B.V.Sc., Veterinary Research Officer, Onderstepoort.

INTRODUCTION.

A progress report on the immunization of horses by the use of formalized virus was published in the Sixteenth Report of the Director of Veterinary Services and Animal Industry. At that time it had been established that a solid immunity can be set up by a series of subcutaneous injections of virulent spleen emulsion inactivated by progressively lower concentrations of formaldehyde. The concentration that will inactivate spleen virus to a degree that renders its injection into fully susceptible horses safe in practically every case was established, namely, 1 : 1000. Even repeated injections of this material did not produce an immunity sufficient to protect against ' O ' virus but horses which are able to resist a vaccine containing 1 : 4000 formaldehyde in the vast majority of instances are immune to O virus. The present series of experiments were planned with the object of proceeding from the 1 : 1000 to the 1 : 4000 material with the smallest number of injections, in the shortest space of time. At the same time a watchful eye had to be maintained on the degree of immunity produced, the keeping qualities of the vaccine, and the possibility of an adverse local effect at the site of injection.

This work was indicated in the hope that sufficiently good results would be obtained to warrant the extension of the method to general immunization in the field in place of the serumvirus simultaneous method which has several serious disadvantages as indicated by Whitworth (1929).

Independent work on similar lines has been carried out by Whitworth and Walker. Generally, the results have been mutually confirmed with this exception, that it must be admitted that the results obtained by us do not warrant that confidence in the efficacy of the method that is apparent in the reports issued from Kenya.

Attention has been directed to the difficulty of producing a bacterially sterile formalized vaccine. Even though the aseptic removal and subsequent manipulation of an organ as bulky as the pathologically enlarged spleen of a reacting horse presents considerable difficulty, the possibility of bacterial contaminants within the spleen itself before removal could not be overlooked. Should any bacteria be present, a final sterile product cannot be anticipated seeing that the only bactericide present in the completed vaccine is formalin in a concentration below its effective limit. No data are available as to the possible harmful effect of bacterial decomposition on the antigenic property of the formalized vaccine but, on several occasions injections have been followed by alarming local reactions and swellings accompanied by such extensive necrosis of the subcutaneous and muscular tissue as to necessitate extensive surgical treatment.

Two methods of sterilization were considered :

(1) The addition of 0·25 per cent. phenol to the saline used for preparing the emulsion.

(2) Filtration of the completed vaccine through a Seitz filter before bottling.

EXPERIMENTAL WORK.

EXPERIMENT 12 (S. 4111a).

Object.—To ascertain the effect on the antigenic property of spleen virus, of (1) adding phenol as a preservative, and (2) of filtration through a Seitz filter.

Method.—The method of vaccine production in this and subsequent experiments was identical with that described in the previous publications. Before use the Seitz filter was autoclaved for 30 minutes at 130°C. under positive pressure. The material was first clarified by aspiration through a thin layer of paper pulp placed over muslin on a Buchner funnel and was then quickly sucked through the filter under a negative pressure of 40 ℔. per square inch. Immediately before filtration a small quantity of a fresh culture of *B. prodigiosus* was added and sterility tests aerobically and anaerobically were carried out on the filtrate : in every case the filtrate was found to be sterile. The actual time of filtration of the large bulk of material was approximately 1½ hours.

At the same time advantage was taken of this experiment to confirm a previous observation that a commencing injection of 1 : 1500 formalized vaccine is dangerous : incidentally the results would also indicate whether the phenol would have any additional attenuating effect upon the virus.

Group A.—This serves as a control group to indicate the efficacy of the particular batch of vaccine. Of the four horses, two died on the 7th day after the 1 : 2000 vaccine. Of the survivors one reacted severely and one mildly to an immunity test of ‘ O ’ virus.

The seven horses in *Group B* received the vaccine to which phenol had been added, five commencing with a 1 : 1500 injection and two with a 1 : 2000 injection ; the latter two horses died on the 6th and 7th day respectively. Of the five that commenced with 1 : 1500, two died as a result of this injection, and 1 as a result of the 1 : 3000 material (third injection). The two survivors at no time reacted, and on immunity test were immune to ‘ O ’ virus.

Results.—Details of the injections together with the results obtained are shown in Table I.

TABLE 1.—*Experiment* 4111 (*a*).

Group.	D.O.B. No. of Horses.	Concentration of formaldehyde in spleen emulsion. 1 : 1500 Date and Dose. 30 c.c.	1 : 2000 Date and Dose. 30 c.c.	1 · 3000 Date and Dose. 30 c.c.	1 : 4000 Date and Dose. 30 c.c.	1 : 6000 Date and Dose. 30 c.c.	Interval in Days between Injections.	Result of Immunization.	Immunity Test. Date of Injection of O-virus.	Interval in days since last Injection.	RESULT.
A. Injection of spleen emulsion.	19800	30/1 30	13 2 30	27/2 30	13 3 30	27 3 30	14	No reaction..	10/4/'30	14	Mild febrile reaction 4th to 8th day.
	19732	30, 1 30	13 2 30	27 2 30	13/3 30	27 3 30	14	No reaction..........	10 4 30	14	Severe reaction 6th to 15th day—recovered.
	19731	—	13/2/30	—	—	—	—	Reacted from 4th day and died 7th day after 1 : 2000 injection	—	—	—
	19793	—	13 2 30	—	—	—	—	Reacted 4th day and died 7th day after 1 : 2000 injection	—	—	—
	19734	30 12 30	—	—	—	—	—	Reacted from 3rd day and died 7th day after 1 . 1500 injection	—	—	—
	19795	30/12 30	—	—	—	—	—	Reacted from 4th day and died 7th day after 1 ; 1500 injection			
B. Injection of spleen emulsion to which 0·25 per cent. Phenol was added	19742	20 2 30	6 3 30	21 3 30	8 4 30	25/4 30	14	No reaction..........	9 5 '30	14	Immune.
	19743	20 2 30	6 3 30	21/3 30	—	—	14	Reacted 4th day and died 7th day after 1 : 3000 injection	—	—	—
	19736	13 2 30	27 2 30	—	13 3 30	27 3 30	14	No reaction. Horse had received filtered spleen vaccine 1 : 2000 before 1 : 1500 injection	10/4/30	14	Immune.
	19733	—	30 1 30	—	—	—	—	Reacted 4th day and died 7th day after 1 : 2000 injection	—	—	—
	19797	—	30/1/30	—	—	—	—	Reacted 4th day and died 7th day after 1 : 2000 injection	—	—	—
C. Injection of spleen emulsion that had been passed through Seitz filter	19735	—	20 c.c. 30 1 30	30 c.c. 13 2 30	15 c.c. 28 2 30	20 c.c. 14 3/30	14	Reacted from 4th day and died 7th day after 1 : 6000 injection	—	—	—
	19794	—	20 c.c. 30/1/30	30 c.c. 13/2 30	30 c.c. 27/2 '30	—	14	Reacted from 5th day and died 8th day after 1 : 4000 injection	—	-	—

Two horses in *Group C* received the Seitz filtrate. Both died as a result of the 1 : 4000 vaccine and one as a result of the 1 : 6000 vaccine.

Conclusions.—(1) It is difficult to offer any definite opinion as to the effect on the antigenic value of the spleen emulsion of adding 0·25 per cent. phenol to the saline, since two out of the four horses in the control group died during the immunizing process, and two out of five died in the experimental group. Possibly it may be concluded that the phenol has no harmful effect.

(2) A formaldehyde concentration of 1 : 1500 either in the presence or absence of phenol does not result in a safe vaccine.

(3) Phenol + formalin has no greater attenuating effect than formalin alone.

(4) Seitz filtration of the vaccine probably removes the greater portion of the antigenic fraction of the vaccine, but allows the passage of that virus which has not been inactivated. This result is of considerable interest because it indicates that the action of formaldehyde in decreasing concentration is not merely to inactivate decreasing amounts of virus, i.e., the immunity produced is not alone due to the repeated injection of increasing but still subinfective doses of virus. On the contrary, the action of the formalin probably is to destroy the infectivity of the virus without reducing its antigenic property; this inactivated virus is unable to pass the disc of a Seitz filter under the conditions of the experiment.

This observation on the low antigenic value of Seitz filtered vaccine was subsequently confirmed by a further test on twelve horses, with material prepared for a later series of experiments. Details of the injections are given in Appendix I. It is necessary only to state here that four of the horses survived the injection of 1: 3500 or 1 : 4000 filtered vaccine and two of the four survivors were able to withstand an immunity test of either 5 c.c. of 'O' virus or 30 c.c. of unfiltered 1 : 4000 vaccine.

Previous work has indicated that the value of formalized vaccine is dependent on a high initial concentration of virus in the emulsion prepared. It is known that blood taken at the height of the febrile reaction is infective in as small an amount as ·0001 c.c. It was, therefore, considered that, if the virus found in the circulating blood is capable of being transformed into a potent vaccine its inclusion in future work might increase the value of the product.

EXPERIMENT S. 4111 b.

Object.—To determine the antigenic value of virulent blood treated with decreasing quantities of formalin in the presence or absence of spleen tissue.

Method.—(*a*) A horse was bled from the jugular vein at the height of its reaction to 'O' virus, formalin was added in varying amounts and injections were made as indicated in Table II.

(*b*) The horse was destroyed *in extremis*, the spleen was removed and pulped by passing it through a Latapi mincer. The contained virus was then inactivated by heating to 100°C. for thirty minutes in an Arnold's steam sterilizer on each of three successive days. The spleen tissue was then made up into a 20 per cent. emulsion, but the infective blood [as obtained for (*a*) above] was used to replace the isotonic saline. Formalin was added by the usual technique and injections made as indicated in Table II.

TABLE II.—*Experiment* 4111 *b.*

Group.	D.O.B. No. of Horse.	Concentration of formaldehyde in blood or blood-spleen emulsion.				Interval in Days between Injections.	Result of Immunization.
		1 : 1500 Date and Dose. 30 c.c.	1 : 2000 Date and Dose. 30 c.c.	1 : 3000 Date and Dose. 30 c.c.	1 : 4000 Date and Dose. 30 c.c.		
A. Injection of formalized blood	19738	30/1/30	—	—	—	—	Reacted from 4th day and died 6th day after 1 : 1500 injection.
	19783	30/1/30	—	—	—	—	Reacted from 5th day and died 7th day after 1 : 1500 injection.
	19739	—	30/1/30	13/2/30	27/2/30	14	Reacted from 8th to 11th day after 1 : 2000; and also reacted from 4th day and died on 8th day after 1 : 4000 injection.
B. Injection of formalized blood mixed with spleen to make a 20 per cent. emulsion. Heated for ½-hour on three successive days to 100°C.	19740	30/1/30	—	—	—	—	Too wild to temperature. Died 10th day after 1 : 1500 injection.
	19798	—	30/1/30	—	—	—	Reacted from 2nd day and died 6th day after 1 : 2000 injection.
	19792	—	30/1/30	—	—	—	Too wild to temperature. Died 11th day after 1 : 2000 injection.

Results.—(*a*) Of three horses which received formalized infective blood two died as a result of the injection of 1 : 1500 fluid, and one which had received 1 : 2000 and 1 : 3000 died as result of the 1 : 4000 blood.

(*b*) Of three horses which received the blood virus formalized in the presence of inactivated spleen tissue one died as a result of the 1 : 1500 concentration and two as a result of the 1 : 2000 concentration.

Conclusion.—Although by no means conclusive, it would appear that the virus found in the blood is not of high antigenic value when formalized in the presence or absence of heat inactivated spleen tissue. A considerable amount of additional work would be required definitely to clear up this point but the results obtained with six horses were so discouraging that the risk of sacrificing any more animals could not be justified. This aspect of the work has, therefore, been discontinued.

Up to the present the major portion of the work had been directed towards the elaboration of a method of immunization based on the injection of virus inactivated by progressively decreasing concentrations of formaldehyde. No serious attempt had been made to ascertain the effect of repeated injections of spleen virus inactivated by the same safe formaldehyde concentration. A series of experiments were, therefore, planned to clear up this point. It was decided to make the interval between injections seven days the details of treatment being as follows:—

1. 1 horse (20307) received 4 injections of 1/1250 vaccine in 60 c.c. doses.
2. 1 ,, (20308) ,, 8 ,, 1/1250 ,, ,,
3. 1 ,, (20325) ,, 4 ,, 1/1500 ,, ,,
4. 1 ,, (20327) ,, 8 ,, 1/1500 ,, ,,
5. 1 ,, (20341) ,, 2 ,, 1/1500 ,, 250 c.c. doses.
6. 1 ,, (20340) ,, 1 injection of 1/1500 ,, 500 c.c. dose.

There were no reactions to the injections but the horses given the 250 c.c. and 500 c.c. doses developed local abscesses requiring surgical treatment.

On applying an immunity test after an interval of ten to fourteen days after the last injection, all the horses except 20308, which had received eight injections of 1/1250 vaccine were found to be fully susceptible to the intravenous injection of 5 c.c. of ' O ' virus and died of horse-sickness. The one exception, 20308, showed a very severe reaction but survived.

Conclusion.—The repeated injection of moderate doses of spleen virus inactivated to a safe degree by an adequate concentration of formaldehyde or the single injection of a massive dose of the same vaccine produces very little or no immunity.

Previous work has shown that the lowest limit of safe formaldehyde concentration is 1 : 1000. In addition it had been found that horses which withstand an injection of 1 : 4000 material are subsequently immune to ' O ' virus. The dose used was fixed arbitrarily at 30 c.c. It was, therefore, decided to carry out a further set of experiments with the object:

(1) Of confirming the conclusions drawn as to the effective limits of formaldehyde concentration.

(2) Of determining whether a smaller dose, which would make for greater convenience in practice, would be equally effective.

(3) Of ascertaining the smallest number of injections required to produce immunity with the smallest risk.

(4) Of ascertaining any difference in antigenic value between individual spleens and batches of different pooled spleens.

(5) Of determining the probable duration of immunity produced by formalized vaccines.

Full details of the injections will be found in Appendix II, of which a summary is shown in Table III of the text.

TABLE III.—EXPERIMENT S. 4193.

Group.	No. of Horses in each Group.	Concentration of formaldehyde in spleen emulsion.					Interval in Days between Injections.	Result of Immunization.	Immunity Test.		
		1 : 1000 Dose.	1 : 2000 Dose.	1 : 3000 Dose.	1 . 4000 Dose.	1 · 6000 Dose.			Injection of O-virus.	Interval in days since last Injection.	RESULT.
A1. Spleen emulsion prepared from Horses 19882, 19883 and 19907	7	30 c.c.	30 c.c.	30 c.c.	30 c.c.	—	14	(*a*) One horse reacted after 1 : 3000 injection and died (*b*) Two horses reacted to 1 : 4000 injection and recovered (*c*) Four horses showed no reactions	— 5 c.c. 5 c.c.	— 14 and 168 14, 28, 83, and 168	— (*b*) Both horses proved to be immune. (*c*) Two horses (14 and 168 days interval) proved to be immune. Two horses (83 and 28 days interval) reacted and recovered.
A2...........	2	20 c.c.	20 c.c.	20 c.c.	20 c.c.	—	14	Two horses did not react	5 c.c.	28. 83	Both horses proved to be immune.
A3...........	2	15 c.c.	15 c.c.	15 c.c.	15 c.c.	—	14	One horse reacted and died after 1 : 3000 injection: other horse reacted after 1 : 4000 injection and recovered	5 c.c.	13	Remaining horse proved to be immune.
A4...........	2	10 c.c.	10 c.c.	10 c.c.	10 c.c.	—	—	Two horses did not react	5 c.c.	28 and 168	Two horses reacted and recovered.
A5...........	2	5 c.c.	5 c.c.	5 c.c.	5 c.c.	—	14	One horse died after 1 : 3000 injection; other horse reacted after 1 : 4000 injection and recovered	5 c.c.	13	Remaining horse proved to be immune.
B1. Spleen emulsion prepared from Horse 19882	2	30 c.c.	30 c.c.	30 c.c.	30 c.c.	—	14	One horse died after 1 : 2000 injection and the other reacted to 1 : 3000 injection and recovered	5 c.c.	13	Remaining horse proved to be immune.

TABLE III—(*continued.*)

Group.	No. of Horses in each Group.	Concentration of formaldehyde in spleen emulsion.					Interval in Days between Injections.	Result of Immunization.	Immunity Test.		
		1 : 1000 Dose.	1 : 2000 Dose.	1 : 3000 Dose	1 : 4000 Dose.	1 : 6000 Dose.			Injection of O-virus.	Interval in days. since last Injection.	RESULT.
B2. Spleen emulsion prepared from Horse 19883	2	30 c.c.	30 c.c.	30 c.c.	30 c.c.	—	14	One horse died after 1 : 2000 injection and the other horse did not react	5 c.c.	13	Remaining horse proved to be immune.
B 3. Spleen emulsion prepared from Horse 19907	2	30 c.c.	30 c.c.	30 c.c.	30 c.c.	—	14	Two horse reacted after 1 : 4000 injection, one died and the other recovered	5 c.c.	13	Remaining horse proved to be immune.
C1. Spleen emulsion prepared from Horses 19882, 19883, 19907	3	30 c.c.	—	30 c.c.	30 c.c.	30 c.c.	14	Two horses did not react, one horse reacted after 1 : 4000 injection and recovered	5 c.c.	13, 28, 83	Horses proved to be immune.
C2..........	2	30 c.c.	—	30 c.c.	30 c.c.	—	14	One horse reacted to 1 : 4000 injection and recovered: other horse showed no symptoms	5 c.c.	14	Horses proved to be immune.
C3..........	5	—	30 c.c.	30 c.c.	—	30 c.c.	14	(*a*) Four horses reacted after 1 : 3000 injection of which three died (*b*) The other horse did did not show any symptoms	5 c.c.	14	(*a*) Remaining horse proved to be immune. (*b*) Horse proved to be immune.

Results: Group A 1.—Seven horses received 4·30 c.c. injections of 1 : 1000, 1 : 2000, 1 : 3000 and 1 : 4000 material; one commenced to react from day 11 after the 1 : 3000 injection and died on day 17; after the 1 : 4000 injection one reacted fairly severely from the 6th day to the 10th day, one from the 5th day to the 10th day, but both recovered. The six survivors were solidly immune to 'O' virus, the immunity test being made in different individuals on the 14th, 28th, 83rd and 168th day after the last immunizing injection.

Group A 2.—Two horses which received injections in 20 c.c. amounts showed no reactions, one being immune on the 28th day, one on the 83rd day.

Group A 3.—Two horses each received 15 c.c. doses; one commenced to react on the 10th day after the 1 : 3000 injection and died on the 14th day (a delayed reaction), one did not react and was immune on the 13th day.

Group A 4.—Two horses received 10 c.c. amounts. There were no reactions to the immunizing injections. On immunity test after an interval of 28 days the one horse reacted severely from the 2nd day to the 10th day and recovered; the other horse after an interval of 168 days commenced to react on the 2nd day and died on the 6th day.

Group A 5.—Two horses received 5 c.c. amounts; the one horse commenced to react on the 6th day after the 1 : 2000 injection and died on the 11th day; the other survived immunization and fourteen days later was solidly immune to 'O' virus.

Conclusions.—The results are by no means clear-cut but it would appear to be inadvisable to reduce the dose of vaccine below 20 c.c. This or a larger dose appears to be safe in the vast majority of cases (8 out of 9), and produces an immunity capable of protecting against 'O' virus for as long as 168 days. Doses smaller than 20 c.c. appear to possess a less favourable balance between antigenic non-virulent fraction and living virus and is less safe (4 out of 6 survived). Moreover, the immunity is of shorter duration since one horse reacted severely after 28 days, one horse died after 168, although two survivors were solidly immune after 13 days.

The material used in the above five groups comprised the pooled emulsion from three spleens. The six horses which make up Group B received injections in 30 c.c. amounts of vaccine prepared from each of the individual spleens.

Results.—Two horses received material from each spleen and of each Group B1 and B2 one died as a result of the 1 : 2000 and in Group B3 one died as result of 1 : 4000 vaccine. In addition one horse reacted severely to the 1 : 4000 material but recovered.

Conclusion.—The results are in striking contrast to those obtained in Group A above. It must be concluded definitely that a safer vaccine can be obtained from the pooled mixture of several spleens than from a single spleen.

Concurrently with the above two groups eight horses were given the series of injections indicated in Groups C1, C2 and C3, with the main object of determining whether a better immunity could be set up by completing the course of injections with 1 : 6000 material, and also of determining whether three injections would not be as effective as four. The final injection of 1 : 6000 was not given in the two horses of C2.

Results.—In Group C1 which were commenced with 1 : 1000 vaccine and then received 1 : 3000, 1 : 4000, and 1 : 6000 vaccine all the animals survived and even after 83 days one horse was still solidly immune.

In Group C2 three injections of 1 : 1000, 1 : 3000 and 1 : 4000 were given. Only one of the horses reacted, viz., to 1 : 4000 vaccine but recovered. Both were found to be immune on the 14th day.

In Group C3 which commenced with 1 : 1500 vaccine and then received 1 : 3000 and 1 : 6000 vaccine four hourses reacted severely to 1 : 3000 vaccine and three died. The two survivors were solidly immune.

Conclusion.—(1) There is no evidence that 1 : 6000 vaccine produces an immunity greater than that set up by 1 : 4000.

(2) It is unsafe to commence with a dilution greater than 1 : 1000 and to jump from 1 : 1500 to 1 : 3000 vaccine may be followed by disaster.

Encouraged by the excellent results obtained in Group C1 above, and bearing in mind that it is apparently not necessary to proceed beyond an injection of 1 : 4000 vaccine an experiment was carried out as shown in Tabel IV.

Object of experiment.—(1) To ascertain whether three injections of 1 : 1000, 1 : 2500 and 1 : 4000 vaccine are both safe and adequate.

(2) To collect data on the keeping quality of vaccine.

Method.—A fresh batch of vaccine was prepared from two spleens and injected into the eight horses in Group A while the same material that was used in Group C of the previous experiment was injected into four horses in Group B after being stored at room temperature for slightly more than four months.

Results.—In Group A, five out of the eight horses reacted to 1 : 4000 vaccine ; of these three died. Of the five survivors four were solidly immune but one reacted severely and recovered. In Group B the two horses that received 1 : 1000, 1 : 2500 and 1 : 4000 vaccine both died as a result of the last injection. Of the two horses that had received 1 : 4000 alone one did not react and one died. The non-reactor proved to be immune.

Conclusion.—(1) The three injections of vaccine are not safe but horses which survive 1 : 4000 vaccine are immune.

(2) There appears to be a definite decrease in antigenic potency after storage for four months at room temperature.

(3) The dose of 10 c.c. apparently is not large enough.

The results obtained in this experiment both as regards the keeping quality of the vaccine, the dose, and the efficacy of three injections were so inconsistent with what had been anticipated that it was decided to carry out an experiment on as large a number of horses as were available, in order to obtain some finality.

Object of the experiment.—(1) To confirm the efficacy of four injections of 1 : 1000, 1 : 2000 and 1 : 3000 and 1 : 4000 vaccine.

(2) To obtain data as to the duration of immunity produced by formalized vaccines.

(3) To ascertain if three injections could not be substituted for the four given in (1) above.

(4) To determine of a concentration of formaldehyde higher than 1 : 4000 which would be safer to include in a course of three injections would still produce a solid immunity to O-virus.

TABLE IV.—EXPERIMENT S. 4271.

Group.	D.O.B. No. of Horses.	Concentration of formaldehyde in spleen emulsion.			Interval in Days between Injections.	Result of Immunization.	Immunity Test.		
		1 : 1000 Dose 10 c.c. and Date.	1 : 2500 Dose 10 c.c. and Date.	1 : 4000 Dose 10 c.c. and Date.			Date of Injection of O-virus.	Interval in days since last Injection.	RESULT.
A. Spleen emulsion prepared from Horses 19965, 19787	19890	7/11/30	21/11/30	5/12/30	14	Reacted from 5th day to 12th day after 1 : 4000 injection	19/12/30	14	Immune.
	19891	7/11/30	21/11/30	5/12/30	14	No reaction (too wild to be temperatured)	19/12/30	14	Immune.
	19892	14/11/30	28/11/30	12/12/30	14	Reacted from 6th to 13th day after 1 : 4000 injection	29/12/30	17	Immune.
	19893	14/11/30	28/11/30	12/12/30	14	Reacted from 3rd day and died 7th day after 1 : 4000 injection	—	—	—
	19894	14/11/30	28/11/30	12/12/30	14	Reacted from 5th day and died 8th day after 1 : 4000 injection	—	—	—
	19895	14/11/30	28/11/30	12/12/30	14	No reaction..................	29/12/30	17	Immune.
	19896	14/11/30	28/11/30	12/12/30	14	No reaction..................	29/12/30	17	Reacted from 4th to 10th day and recovered.
	19897	14/11/30	28/11/30	12/12/30	14	Reacted from 3rd day and died 7th day after 1 : 4000 injection	—	—	—
B. Spleen emulsion prepared from Horses 19882, 19883, 19907	19898	14/11/30	28/11/30	12/12/30	14	Reacted from 5th day and died 9th day after 1 : 4000 injection	—	—	—
	19899	14/11/30	28/11/30	12/12/30	14	Reacted from 5th day and died 10th day after 1 : 4000 injection	—	—	—
	19992	—	—	17/12/30	—	No reaction..................	29/12/30	12	Immune.
	19993	—	—	23/12/30	—	Reacted from 4th day and died 7th day after 1 : 4000 injection	—	—	—

Method.—The details of injections together with the results will be found in Appendix III, of which a summary is given in Table V of the text. The vaccine was prepared from three spleens and was pooled before bottling or injection. The dose was fixed at 30 c.c. since the smaller dose given above may in part have been responsible for the bad results.

Results: Group A (*A*1–*A*5).—Ten horses received 1 : 1000, 1 : 2000, 1 : 3000 and 1 : 4000 vaccine at 14 or 21 day intervals as indicated. During this course of immunization one horse reacted very mildly to 1 : 1000 vaccine one reacted mildly to 1 : 2000 vaccine, two reacted severely and one died after 1 : 4000 vaccine, while the remaining five showed no reactions. On immunity test after 14 days, two horses were immune, after 21 days two horses appeared to have acquired either no immunity or to have lost it because both died, the one with a slightly prolonged reaction. After 138 days the reaction to O-virus was that which would have been anticipated in fully susceptible horses. Owing to this loss of immunity the remaining three horses were given virus + hyperimmune serum as practised in the simultaneous method of immunization. Only one horse showed a very mild reaction, although at least two severe reactions would have been anticipated in susceptible animals.

*Group A*6.—Ten horses received a course of injections identical with those given in A1–A5 seven months later, i.e., the vaccine had been stored for seven months at room temperature. On this occasion two horses showed a mild febrile reaction after 1 : 2000 vaccine, two reacted more severely to 1 : 3000 vaccine and of two severe reactors to 1 : 4000 vaccine one died and one recovered. An immunity test with O-virus was made 28 days after the last injection when two died, three showed reactions of varying severity and the remaining five were immune. It is worthy of note that the two deaths and three reactors occurred among six horses that had shown no febrile reaction at any time during the process of immunization. The results are not dissimilar to those obtained in the first instance, the vaccine having been stored for approximately seven months at room temperature.

*Group B*1 *and B*2.—Four horses received 1 : 1000, 1 : 2500 and 1 : 3000 vaccine; two reacted severely to the 1 : 3000 injection one dying and the other being destroyed *in extremis.* Of the two survivors one died on testing the immunity after an interval of 21 days, the other animal was given serum and virus 149 days later and showed a mild reaction.

*Group C*1–*C*3.—Four horses received 1 : 1000, 1 : 2500 and 1 : 4000 vaccine, three horses showed no reaction, one reacting severely to 1 : 3500 vaccine and was found to be immune 71 days later. After an interval of 21 days, of the three non-reactors two were found to possess little immunity to O-virus (one died, one reacted severely and recovered); the other was given serum and virus after 149 days and showed no clinical reaction.

*Group D*1–*D*3.—Nine horses received 1 : 1000, 1 : 2500 and 1 : 4000 vaccine; five horses did not react, but four reacted severely to 1 : 4000 vaccine and one died, the survivor being solidly immune after 21 days. Of the non-reactors two showed a severe reaction to O-virus after 21 days but recovered; one died after 146 days; three received serum and virus after 149 days, one showing no reaction.

Group E.—One horse received 1 : 1000, 1 : 2500, 1 : 3000 and 1 : 4000 vaccine. There was no reaction during immunization but O-virus after 14 days produced Dikkop horse-sickness though the animal recovered.

Group F1–F7.—Served as controls for the infectivity of the various formalin dilutions. It will be seen that there was sufficient free virus in 30 c.c. of 1 : 3000, 1 : 3500 and 1 : 4000 vaccine to set up fatal infections in horses. In 1 : 1000 and 1 : 2000 the amount would appear to be on the border line of a single infective dose, since three horses did not react; several horses in the experimental groups, however, undoubtedly reacted to 1 : 2000.

Conclusions.—(1) Possibly the most striking conclusion to be drawn from this experiment is the great variation in antigenic potency of different batches of pooled spleens even though the greatest care is taken to standardize each process in the production of the vaccine. In this experiment it would appear that the vaccine was of low potency, since one horse died after 1 : 4000 injection, and though two horses were immune after 14 days, an immunity sufficient to withstand 5 c.c. of O-virus had disappeared after 28 days. However, even after 149 days there must have been some residual immunity since survivors which received serum and virus reacted at most with a mild fever whereas a mortality of 5 per cent. and a fairly severe reaction in the majority of horses would not have been surprising.

(2) The keeping quality of even a poor batch of vaccine is good since after storage for approximately seven months at room temperature practically no difference in potency could be ascertained in two groups of ten horses.

(3) A formaldehyde concentration of 1 : 1000 is safe as a commencing dose.

(4) It must be concluded that only with a vaccine of high potency are survivors of a 1 : 4000 injection immune to O-virus.

(5) A previous observation that immunity produced by formalized vaccine may last for at least 168 days must be modified, since in this experiment there was a marked decrease in immunity after 28 days though some immunity could be detected after 142 days.

(6) There appears to be a better immunity produced in those animals which show a definite clinical reaction during immunization than in animals which show no reaction.

(7) To complete a series of injections with a formaldehyde concentration greater than 1 : 4000 results in an immunity of lower grade.

(8) The best results are obtained with four injections of 1 : 1000, 1 : 2000' 1 : 3000 and 1 : 4000 vaccine.

(9) Apart from exceptional vaccines of high potency the possibility of reducing the number of vaccinations from four to three does not appear to be good.

(10) The best results obtained with three injections were with 1 : 1000, 1 : 2500 and 1 : 4000 vaccine. Making the second of three injections either 1 : 2000 or 1 : 3000 is not safe.

At this stage attention must be directed to the fact that the injection of 5 c.c. of O-virus intravenously is an exceeding severe immunity test, possibly more severe than a test of immunity by exposure to natural infection. No data are available to substantiate this expression of opinion, however, since the transmitter of the virus under field conditions is not known. Still, horses which have received O-virus together with hyperimmune serum have reacted in a manner certainly indicative of the presence of some immunity. Consequently, it was decided to run a further series of experiments to ascertain whether it would not be possible to combine the simultaneous serum virus method with the formalized spleen virus method in the hope:—

(1) That the amount of serum required for each injection could be considerably reduced.

(2) That the margin of safety of the formalized vaccine method might be increased.

(3) That the danger of the consolidating dose of virus might be minimized by the injection of serum simultaneously.

TABLE V.—EXPERIMENT S. 4496.

Group.	No. of Horses in each Group.	Concentration of formaldehyde in spleen emulsion.											Result of Immunization.	Interval in Days since last Injection.	Injection of hyperimmune serum and N.-virus.	Inter-val in Days.	Injection of hyperimmune serum and O.-virus.	Injection of O.-virus.	RESULT.
		1 : 1000 Dose.	Inter-val in Days.	1 : 2000 Dose.	Inter-val in Days.	1 : 2500 Dose.	Inter-val in Days.	1 : 3000 Dose.	Inter-val in Days.	1 : 3500 Dose.	Inter-val in Days.	1 : 4000. Dose.							
A1	2	30 c.c.	14	30 c.c.	—	—	14	30 c.c.	—	—	14	30 c.c.	One horse showed a mild febrile reaction after 1 : 1000 injection and the other showed no symptoms	21	—	—	—	5 c.c.	Both horses died from horse-sickness.
A2	2	30 c.c.	14	30 c.c.	—	—	14	30 c.c.	—	—	14	30 c.c.	No reactions observed........	138	—	—	—	5 c.c.	Both horses died from horse-sickness.
A3	1	30 c.c.	14	30 c.c.	—	—	14	30 c.c.	—	—	14	30 c.c.	Febrile reaction after 1 : 2000 injection	142	400 + 5 c.c.	3	400 + 5 c.c.	—	Very mild febrile horse-sickness reaction and recovered.
A4	3	30 c.c.	14	30 c.c.	—	—	14	30 c.c.	—	—	21	30 c.c.	Two horses reacted after 1 : 4000 injection, and recovered. One horse died after 1 : 4000 injection.	14	—	—	—	5 c.c.	Remaining two horses proved to be immune.
A5	2	30 c.c.	14	30 c.c.	—	—	14	30 c.c.	—	—	21	30 c.c.	No reactions observed........	136	400 + 5 c.c.	3	400 + 5 c.c.	—	Horses proved to be immune.
A6	10	30 c.c.	14	30 c.c.	—	—	21	30 c.c.	—	—	21	30 c.c.	(*a*) No reactions observed in four horses (*b*) Febrile reactions observed in two horses after 1 : 2000 injection (*c*) Febrile reactions observed in two horses after 1 : 3000 injection (*d*) Febrile reactions observed in two horses after 1 : 4000 injection, one of which died	28	—	—	—	5 c.c.	(*a*) One horse reacted and died from horse-sickness. Two horses reacted and recovered. One horse proved to be immune. (*b*) One horse reacted and recovered. One horse reacted and died from horse-sickness. (*c*) Both horses proved to be immune. (*d*) Remaining horse proved to be immune.
B1	3	30 c.c.	—	—	14	30 c.c.	21	30 c.c.	—	—	—		(*a*) Mild febrile reaction observed in one horse after 1 : 1000 injection (*b*) Two horses showed reactions after 1 : 3000 injection; one horse died and the other was killed *in extremis*	21	—	—	—	5 c.c.	(*a*) Horse died from horse-sickness.
B2	1	30 c.c.	—	—	14	30 c.c.	21	30 c.c.	—	—	—	—	No reaction observed.........	140	400 + 5 c.c.	3	400 + 5 c.c.	—	Mild febrile horse-sickness reaction and recovered.
C1	2	30 c.c.	—	—	14	30 c.c.	—	—	21	30 c.c.	—	—	No reactions observed........	21	—	—	—	5 c.c.	Both horses reacted to horse-sickness. One recovered and one died.
C2	1	30 c.c.	—	—	14	30 c.c.		—	21	30 c.c.	—	—	Reacted after 1 : 3500 injection and recovered	71	—		—	5 c.c.	Horse proved to be immune.
C3	1	30 c.c.	—	—	14	30 c.c.	—	—	21	30 c.c.	—	—	No reaction observed.........	149	400 + 5 c.c.	3	400 + 5 c.c.	—	Horse proved to be immune.
D1	4	30 c.c.	—	—	14	—	—	—	—	—	21	30 c.c.	(*a*) One horse showed no reaction (*b*) Two horses reacted after 1 : 4000 injection, one of which died (*c*) One horse showed febrile reaction after 1 : 1000	21	—	—	—	5 c.c.	(*a*) Mild febrile reaction and recovered. (*b*) Horse proved to be immune. (*c*) Reacted severely and recovered.
D2	2	30 c.c.	—	—	14	30 c.c.	—	—	—	—	21	30 c.c.	No reactions observed........	145	—	—	—	5 c.c.	One horse proved to be immune and the other died from horse-sickness.
D3	3	30 c.c.	—	—	14	30 c.c.	—	—	—	—	21	30 c.c.	(*a*) One horse did not react... (*b*) Two horses reacted after 1 : 4000 injection and recovered	149	400 + 5 c.c.	3	400 + 5 c.c.	—	Three horses proved to be immune.
E1	1	30 c.c.	—	—	14	30 c.c.	21	30 c.c.	—	—	41	30 c.c.	No reaction observed.........	15	—	—	—	5 c.c.	Reacted severely and recovered.
F1	1	—	—	30 c.c.	—	—	—	—	—	—	—	—	No reaction observed.........	21	—	—	—	5 c.c.	Died from horse-sickness.
F2	1	—	—	—	—	30 c.c.	—	—	—	—	—	—	No reaction observed.........	21	—	—	—	5 c.c.	Died from horse-sickness.
F3	2	—	—	—	—	—	—	30 c.c.	—	—	—	—	One horse showed no reaction, and the other died	26	—	—	—	—	Died from horse-sickness.
F4	1	—	—	—	—	—	—	—	—	30 c.c.	—	—	Died from horse-sickness......	—	—	—	—	—	—
F5	1	—	—	—	—	—	—	—	—	—	—	30 c.c.	Died from horse-sickness......	—	—	—	—	—	—
F6	1	—	—	30 c.c.	—	—	—	—	—	—	22	30 c.c.	No reaction..................	20	—	—	—	5 c.c.	Died from horse-sickness.
F7	1	—	—	—	—	30 c.c.	—	—	—	—	22	30 c.c.	Died from horse-sickness......	—	—	—	—	—	—

A summary of the injections in t[illegible] T[illegible], while full details of the reactions to each injection are given in Appendix IV.

TABLE VI.

Group.	No. of Horses.	Concentration of formaldehyde in spleen emulsion. 1 : 1000 Dose.	Interval in Days.	1 : 2000 Dose.	Interval in Days.	1 : 3000 Dose.	Interval in Days.	1 : 4000 Dose.	Interval in Days.	N.-virus + Hyper-immune serum Dose	Interval in Days	O.-virus + Hyper-immune serum Dose.	Interval in Days.	O.-virus. Dose.	RESULT.
I.	10	—	—	—	—	—	—	—	—	5 + 400 c.c.	3	5 + 400 c.c.	—	—	Two horses died; eight showed severe reactions.
II.	10	60 c.c.	—	—	—	—	—	—	14	5 + 400 c.c.	3	5 + 400 c.c.	—	—	Following serum + virus two horses died, five reacted severely but recovered, two reacted mildly and one did not react.
III.	2	60 c.c.	—	—	—	—	—	—	14	5 + 200 c.c.	3	5 + 200 c.c.	—	—	One horse showed a moderate reaction, the other a mild reaction.
IV.	7	60 c.c.	—	—	14	30 + 400 c.c. serum	—	—	—	—	3	5 + 400 c.c.	—	—	Three horses showed severe and four moderate reactions. All recovered.
V.	2	60 c.c.	—	—	14	30 + 200 c.c. serum	—	—	—	—	3	5 + 200 c.c	—	—	Both horses reacted severely but recovered.
VI.	2	60 c.c.	14	60 c.c.	—	—	—	—	—	—	14	5 + 400 c.c.	—	—	One horse reacted severely and recovered; one showed no reaction.
VII.	2	60 c.c.	14	60 c.c.	—	—	—	—	—	—	14	5 + 200 c.c.	—	—	One horse succumbed to the 1 : 1000 formalized spleen emulsion, the other died after serum-virus injection.
VIII.	2	60 c.c.	14	60 c.c.	14	60 c.c.	—	—	—	—	14	5 + 200 c.c.	—	—	One horse reacted severely, the other mildly.
IX.	2	30 c.c.	14	30 c.c.	14	30 c.c.	—	—	—	—	14	5 + 200 c.c.	—	—	One horse died the other reacted severely but recovered.
X.	5	30 c.c.	14	30 c.c.	14	30 c.c.	14	30 c.c.	—	—	14	5 + 400 c.c.	—	—	Three reacted mildly; two did not react; one horse reacted severely after 1 : 4000 injection.
XI.	5	30 c.c.	14	30 c.c.	14	30 c.c.	14	30 c.c.	—	—	14	5 + 200 c.c.	—	—	One horse died, two reacted, severely, one reacted mildly one did not react.
XII.	5	30 c.c.	14	30 c.c.	14	30 c.c.	14	30 c.c.	—	—	—	—	14	5 c.c.	Two horses died, two reacted severely, one reacted mildly.

It will be observed that the experiment was planned in such a way as to vary from a group of horses immunized by the simultaneous serum virus method to a group of horses immunized by what is considered the safest and most efficient formalized vaccine method, namely, four injections of 1 : 1000, 1 : 2000, 1 : 3000, 1 : 4000 vaccine at 14 day intervals. Intermediate groups were included to constitute a gradation from the one extreme to the other, and at the same time to attempt to reduce the amount of hyperimmune serum necessary to control the reactions to the fully virulent virus.

Results.—Group I.—Of the ten horses immunized by the serum virus method two horses died of Dikkop horse-sickness on the 12th and 13th day respectively, while the remaining eight recovered after undergoing severe clinical reactions.

Group II.—When 60 c.c. of 1 : 1000 vaccine was given as a preliminary immunizing injection 14 days prior to the serum virus method the result was slightly better, though the percentage mortality was the same, i.e., two out of ten died, one horse showed no symptoms, one reacted mildly and five survived severe reactions.

Group III.—This group differed from II above only in respect of the dose of serum which was reduced by half to 200 c.c.: both horses survived, one after a severe, the other after a mild reaction.

Group IV.—A preliminary injection of 60 c.c. of 1 : 1000 vaccine was given, but the " N " virus in the first of the serum virus injections was replaced by 1 : 3000 vaccine a " strength " of vaccine to which at least a very severe reaction would have been expected. Out of seven horses three showed mild reaction, and four reacted severely but recovered.

Group V.—This group differed from IV above by the reduction of the dose of serum to half. Of two horses, both reacted severely but recovered.

Groups VI and VII received two injections of vaccine (1 : 1000 and 1 : 2000) followed by O-virus + serum. When the dose of the serum was 400 c.c. both horses recovered, although one reacted severely. When the dose of serum was 200 c.c. the one horse that survived vaccination died.

Groups VIII and IX, received three injections of vaccine (1 : 1000, 1 : 2000, 1 : 3000) followed by O-virus and a half dose of serum. When the dose of vaccine was 60 c.c. both horses recovered, although one reacted severely. When the dose was 30 c.c. one reacted severely and recovered, the other died.

Groups X, XI, and XII received the ordinary course of four formalized spleen injections but five horses received a consolidating or immunity test injection of O-virus, five received O-virus + half dose of hyperimmune serum, and five received O-virus + a full dose of hyperimmune serum. Only one horse showed a reaction to vaccination. When O-virus alone was given two horses died, one recovered after a severe reaction, and two reacted mildly. With the addition of 200 c.c. of serum, one died, two reacted severely, one mildly and one was solidly immune. But with 400 c.c. of serum there were no deaths, three reacted very mildly and two showed no clinical disturbance.

Conclusions.—A direct comparison of the two methods of immunization is possible from this series of experiments, though it must be stated that the results obtained with the serum virus method were not good since the reactions were more severe and the mortality was higher than usually seen in the field.

On the other hand, the antigenic value of the batch of three pooled formalized spleens used was poor since two out of five horses which survived the 1 : 4000 injection developed an immunity insufficient to protect against 5 c.c. of O-virus intravenously.

The four injections of formalized vaccine caused a severe reaction in only one out of fifteen horses, but a degree of immunity was produced which could protect against O-virus only when modified by the simultaneous injection of a massive dose of hyperimmune serum. Previous work has shown that formalized virus sets up an immunity which may decrease to a low level is as short a time as 28 days so that the consolidating dose of virus must be regarded not as an immunity test but as an integral part of the prophylactic treatment. Consequently, unless massive doses of hyperimmune serum are used the formalized vaccine method has no advantage over the serum virus method in respect of safety. No data are available as to the incidence of alarming sequelae such as staggers, but in view of the necessity for a massive dose of serum and the suspected relationship between large doses of serum and the incidence of staggers, any advantage in this respect is doubtful. A comparison between the degree of protection to natural infection in the field could only be made after several seasons exposure of immunized horses, but since a single virus has been used in the vaccine method, and since two antigenically different strains are used in the serum virus method, it is not anticipated that any advantage will rest with the vaccine method.

For the rest it would appear that no striking results can be expected from a more intimate fusion or combination of the two methods.

DISCUSSION.

The previous observation that horses may be immunized by a series of injections of spleen virus inactivated by progressively decreasing concentrations of formaldehyde has been confirmed. When due regard is paid to the practical necessity of reducing the number of injections to the minimum commensurate with safety and efficacy, the best results have been obtained with four injections of spleen virus inactivated by concentrations of 1 : 1000, 1 : 2000, 1 : 3000, and 1 : 4000 formaldehyde. To commence immunization with a vaccine containing a formaldehyde concentration less than 1 : 1000 is definitely dangerous; repeated injections of 1 : 1000 vaccine has been shown previously not to produce an immunity of high order, but horses which survive a 1 : 4000 vaccine after suitable preliminary treatment are in the vast majority of instances solidly immune to O-virus. The progression from 1 : 1000 to 1 : 4000 vaccine must be slow and gradual if the danger of breakdowns to horse-sickness is to be avoided.

The antigenic value of different individual spleens varies within wide limits. To obtain a final product of high potency it is essential to pool the material prepared from three spleens at the very least. Preferably batches of vaccine should be made up to comprise the spleens of a greater number of horses, since the vaccine from three has been shown to vary greatly in value.

Phenol in a concentration of 0·25 per cent. may be added as a preservative either before or after formalizing as it appears to have no detrimental effect upon the potency of the vaccine. At the same time it must be remembered that phenol neither increases nor decreases the attenuating action of formalin. Filtration through Seitz discs as an aid to the production of a sterile vaccine is definitely contra-indicated since the living virus appears partly to pass through with the filtrate while the avirulent antigenic portion is retained.

When the potency of a particular batch of vaccine is higher than the average the immunity produced may be of considerable duration, it has been proved to be of a high order, as long as six months after the last injection. On the other hand, even after as short an interval as 28 days immunity has been found to fall to such a level that it was unable to protect against O-virus. Consequently, the injection of fully virulent virus should be regarded not so much as an immunity test but rather as an integral portion of the process of immunization. Exposure to nature infection may be considered as an alternative, but in South Africa the seasonable occurrence of horse-sickness is so variable and so irregular, that, in the absence of any definite knowledge of the natural transmitter, this procedure is too uncertain to be of practical value.

In all the work reported here the strain of virus which has been used is O-virus. This strain has been maintained in horses by serial passage for more than 250 generations over a period of approximately twenty-five years. Consequently, due consideration should be given to the possibility of this virus having become 'fixed' for horses without diminition of antigenic value. If this is conceded it will indicate that the injection of O-virus with its enhanced infectivity constitutes too serious a test on the immunity of the horse. This is borne out by the observation that, if a dose of hyperimmune serum is given simultaneously with the virus the reaction is almost completely blocked, although this would certainly not be the case with fully susceptible horses. There exists a possibility that a similar end result would be obtained if a less virulent and antigenically similar strain of virus was used for the consolidating injection. If it is found to be essential to use hyperimmune serum then one of the chief advantages of the formalized vaccine disappears.

On the whole the results with formalized vaccine have been disappointing. The margin of safety appears to be too narrow to permit of application of the method to the field on a large scale. But what is of equal importance is the fact that by the methods investigated so far there does not appear to be a reasonable possibility of advancing to the production of a polyvalent vaccine, so that the number of subsequent breakdowns cannot be less than is experienced by the present simultaneous serum virus method. Consequently, it must be concluded reluctantly that in the present state of our knowledge the application of the formalized spleen virus method holds out little hope of solving many of the difficulties encountered in the immunization of horses against horse-sickness.

SUMMARY.

(1) The antigenic value of individual spleens varies greatly.

(2) The product of several pooled spleens is more potent than that obtained from a single spleen.

(3) The potency of different batches of pooled spleens varies.

(4) The keeping quality of formalized vaccines for four months is good. After that time there is evidence of decrease in value (not tested after seven months).

(5) The addition of phenol as a bactericide has no detrimental effect.

(6) Filtration through a Seitz filter is contra-indicated.

(7) The lowest concentration of formaldehyde required to produce a safe vaccine is 1 : 1000.

(8) The lowest concentration of formaldehyde able to produce a solid immunity to O-virus is 1 : 4000.

(9) It is not safe to proceed from a 1 : 1000 vaccine to a 1 : 4000 vaccine n less than two intermediate steps.

(10) The best results have been obtained with four injections of 1 : 1000, 1 : 2000, 1 : 3000 and 1 : 4000 vaccine.

(11) The interval between injections should not be less than 14 days, or greater than 21 days.

(12) The dose of vaccine should not be smaller than 20 c.c. A dose of 30 c.c. has given the most constant results.

(13) The immunity produced by the vaccine may last for as long as six months. Usually it is transient and on accasion has markedly decreased in 28 days.

(14) It is necessary to complete immunization by a dose of fully virulent virus.

(15) The margin of safety of the method is narrow.

(16) The application of the method to the field cannot be recommended in the present state of our knowledge.

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APPENDIX I.—EXPERIMENT 4351.

Group.	D.O.B. No. of Horses.	Concentration of formaldehyde in spleen emulsion. Material passed through Seitz filter before injection.											Result of Immunization.	Immunity Test.		
		1 : 1000 Dose 30 c.c. and Date.	Interval in Days.	1 : 2000 Dose 30 c.c. and Date.	Interval in Days.	1 : 2500 Dose 30 c.c. and Date.	Interval in Days.	1 : 3000 Dose 30 c.c. and Date.	Interval in Days.	1 : 3500 Dose 30 c.c. and Date.	Interval in Days.	1 : 4000 Dose 30 c.c. and Date.		Date of Injection of O.-virus.	Interval in days since last Injection.	RESULT.
A.	20054	10·2·31	14	24·2·31	—	—	14	10·3·31	—	—	21	31·3·31	Reacted from 5th day and died 9th day after 1 : 4000 injection	—	—	—
	20055	10·2·31	14	24·2·31	—	—	14	10·3·31	—	—	21 28	31·3·31 30 c.c. unfiltered 28·4·31	Reacted from 15th to 20th day after 1 : 2000 injection	13·5·31	14	Severe reaction 3rd to 11th day and recovered.
	20056	10·2·31	14	24·2·31	—	—	14	10·3·31	—	—	21 28	31·3·31 30 c.c. unfiltered 28·4·31	No reaction............. Reacted 5th day and died 8th day after 1 : 4000 injection	—	—	—
B.	20057	10·2·31	—	—	14	24·2·31	—	—	21	17·3·31	—	—	Reacted from 6th day and died 8th day after 1 : 3500 injection	—	—	—
	20058	10·2·31	—	—	14	24·2·31	—	—	21	17·3·31	—	—	Reacted from 6th day and died 9th day after 1 : 3500 injection	—	—	—
	20059	10·2·31	—	—	14	24·2·31	—	—	21	17·3·31	—	—	Reacted 6th day and died 9th day after 1 : 3500 injection	—	—	—
C.	20060	10·2·31	—	—	14	24·2·31	21	17·3·31	—	—	22	8·4·31	Reacted from 8th day and died 13th day after 1 : 4000 injection	—	—	—
	20061	10·2·31	—	—	14	24·2·31	21	17·3·31	—	—	22	8·4·31	Reacted from 5th day and died 9th day after 1 : 4000 injection	—	—	—
	20062	10·2·31	—	—	14	24·2·31	21	17·3·31	—	—	22	8·4·31	Reacted from 6th day and died 9th day after 1 : 4000 injection	—	—	—
D.	20063	10·2·31	—	—	14	24·2·31	—	—	—	—	21	17·3·31	Severe reaction 6th to 11th day after 1 : 4000 injection	8·4·31	22	Reacted from 3rd day and died 6th day.
	20064	10·2·31	—	—	14	24·2·31	—	—	—	—	21	17·3·31	Reacted from 2nd day to 7th day after 1 : 2500 injection	8·4·31	22	Severe reaction 3rd to 9th day and recovered.
	20065	10·2·31	—	—	14	24·2·31	—	—	—	—	21	17·3·31	Reacted from 4th day and died 8th day after 1 : 4000 injection	—	—	—

APPENDIX II.—EXPERIMENT 4193.

Group.	D.O.B. No. of Horses.	Concentration of formaldehyde in spleen emulsion. 1 : 1000 Dose and Date.	1 : 1500 Dose and Date.	1 : 2000 Dose and Date.	1 : 3000 Dose and Date.	1 : 4000 Dose and Date.	1 : 6000 Dose and Date.	Interval in days between Injections.	Result of Immunization.	Immunity Test. Date of Injection of O. virus.	Interval in days since last Injection.	RESULT.
A1. Spleen emulsion prepared from Horses 19907, 19883, 19882	19853	30 c.c. 9/7/30	—	30 c.c. 22·7·30	30 c.c. 6/8/30	30 c.c. 21·8·30	—	14	Reacted severely from 6th to 10th day after 1 : 4000 injection	3/9/30	14	Proved to be immune.
	19854	30 c.c. 9/7/30	—	30 c.c. 22·7·30	30 c.c. 6/8/30	30 c.c. 21·8·30	—	14	No reaction (too wild to be temperatured)	3/9/30	14	Proved to be immune.
	19856	30 c.c. 9/7/30	—	30 c.c. 22·7·30	30 c.c. 6/8/30	30 c.c. 21·8·30	—	14	No reaction (too wild to be temperatured)	18/9/30	28	Reacted from 5th to 10th day and recovered.
	19857	30 c.c. 9/7/30	—	30 c.c. 22·7·30	30 c.c. 6/8/30	30 c.c. 21·8·30	—	14	No reaction (too wild to be temperatured)	12/11/30	83	Reacted from 4th to 8th day and recovered.
	19858	30 c.c. 9/7/30	—	30 c.c. 22·7·30	30 c.c. 6/8/30	30 c.c. 21·8·30	—	14	No reaction (too wild to be temperatured)	4/2/31	168	Proved to be immune.
	19859	30 c.c. 9/7/30	—	30 c.c. 22·7·30	30 c.c. 6/8/30	30 c.c. 21·8·30	—	14	Reacted from 11th and died 17th day after 1 : 3000 injection	—	—	—
	19860	30 c.c. 9/7/30	—	30 c.c. 22·7·30	30 c.c. 6/8/30	30 c.c. 21·8·30	—	14	Reacted severely from 5th to 10th day after 1 : 4000 injection and recovered	4/2/31	168	Proved to be immune.
A2.	19861	20 c.c. 9/7/30	—	20 c.c. 22·7·30	20 c.c. 6/8/30	20 c.c. 21·8·30	—	14	No reaction (too wild to be temperatured)	18/9/30	28	Proved to be immune.
	19862	20 c.c. 9/7/30	—	20 c.c. 22·7·30	20 c.c. 6/8/30	20 c.c. 21·8·30	—	14	No reaction (too wild to be temperatured)	12/11/30	83	Proved to be immune.
A3.	19863	15 c.c. 9/7/30	—	15 c.c. 22·7·30	15 c.c. 6/8/30	—	—	14	Reacted from 11th and died 14th day after 1 : 3000 injection	—	—	—
	19864	15 c.c. 9/7/30	—	15 c.c. 22·7·30	15 c.c. 6/8/30	15 c.c. 21·8·30	—	14	Mild reaction 6th to 12th day after 1 : 4000 injection	3/9/30	13	Proved to be immune.
A4.	19865	10 c.c. 9/7/30	—	10 c.c. 22·7·30	10 c.c. 6/8/30	10 c.c. 21·8·30	—	14	No reaction..................	18/9/30	28	Reacted from 3rd to 9th day and recovered.
	19866	10 c.c. 9/7/30	—	10 c.c. 22·7·30	10 c.c. 6/8/30	10 c.c. 21·8·30	—	14	No reaction..................	4/2/31	168	Reacted from 2nd and died on 6th day.
A5.	19867	5 c.c. 9/7/30	—	5 c.c. 22·7·30	5 c.c. 6/8/30	—	—	14	Reacted from 7th and died on 11th day after 1 : 3000 injection	—	—	—
	19868	5 c.c. 9/7/30	—	5 c.c. 22·7·30	5 c.c. 6/8/30	5 c.c. 21·8·30	—	14	Reacted from 8th to 16th day after 1 : 4000 injection	3/9/30	13	Proved to be immune.

APPENDIX II—(*continued.*)

Group.	D.O.B. No. of Horses.	Concentration of formaldehyde in spleen emulsion.						Interval in days between Injections.	Result of Immunization.	Immunity Test.		
		1 : 1000 Dose and Date.	1 : 1500 Dose and Date.	1 : 2000 Dose and Date.	1 : 3000 Dose and Date.	1 : 4000 Dose and Date.	1 : 6000 Dose and Date.			Date of Injection of O.-virus.	Interval in days since last Injection.	RESULT.
B1. Spleen emulsion prepared from Horse 19882	19869	30 c.c. 9/7/30	—	30 c.c. 22·7·30	—	—	—	14	Reacted from 6th and died on 9th day after 1 : 2000 injection	—	—	—
	19870	30 c.c. 9/7/30	—	30 c.c. 22·7·30	30 c.c. 6·8·30	30 c.c. 21·8·30	—	14	Reacted from 5th to 12th day after 1 : 3000 injection	3/9/30	13	Proved to be immune.
B2. Spleen emulsion prepared from Horse 19883	19873	30 c.c. 9/7/30	—	30 c.c. 22·7·30	30 c.c. 6/8/30	30 c.c. 21·8·30	—	14	No reaction.................	3/9/30	13	Proved to be immune.
	19874	30 c.c. 9/7/30	—	30 c.c. 22·7·30	30 c.c. 6·8·30	—	—	14	Reacted from 7th and died 17th day after 1 : 2000 injection	—	—	—
B3. Spleen emulsion prepared from Horse 19907	19875	30 c.c. 9/7/30	—	30 c.c. 22·7·30	30 c.c. 6·8·30	30 c.c. 21·8·30	—	14	Reacted from 6th and died 8th day after 1 : 4000 injection	—	—	—
	19876	30 c.c. 9/7/30	—	30 c.c. 22·7·30	30 c.c. 6·8·30	30 c.c. 21·8·30	—	14	Reacted from 6th to 13th day after 1 : 4000 injection	3/9/30	13	Proved to be immune.
C1. Spleen emulsion prepared from Horses 19882, 19883, 19907	19885	30 c.c. 9/7/30	—	—	30 c.c. 22·7·30	30 c.c. 6·8·30	30 c.c. 21·8·30	14	No reaction.................	3/9/30	13	Proved to be immune.
	19886	30 c.c. 9/7/30	—	—	30 c.c. 22·7·30	30 c.c. 6/8/30	30 c.c. 21·8·30	14	Reacted from 12th to 18th day after 1 : 4000 injection	18/9/30	28	Proved to be immune.
	19887	30 c.c. 9/7/30	—	—	30 c.c. 22·7·30	30 c.c. 6·8·30	30 c.c. 21·8·30	14	No reaction. Too wild to be temperatured	12/11/30	83	Proved to be immune.
C2.	19888	30 c.c. 9/7/30	—	—	30 c.c. 22·7·30	30 c.c. 6·8·30	—	14	No reaction. Too wild to be temperatured	20/8/30	14	Proved to be immune.
	19889	30 c.c. 9/7/30	—	—	30 c.c. 22·7·30	30 c.c. 6·8·30	—	14	Reacted from 5th to 11th day after 1 : 4000 injection	20/8/30	14	Proved to be immune.
C3.	19877	—	30 c.c. 9/7/30	—	30 c.c. 22·7·30	—	30 c.c. 6/8/30	14	Reacted from 15th to 23rd day after 1 : 3000 injection	20/8/30	14	Proved to be immune.
	19878	—	30 c.c. 9/7/30	—	30 c.c. 22·7·30	—	30 c.c. 6/8/30	14	Reacted from 6th and died 9th day after 1 : 3000 injection	—	—	—
	19879	—	30 c.c. 9/7/30	—	30 c.c. 22·7·30	—	30 c.c. 6/8/30	14	No reaction......	20/8/30	14	Proved to be immune.
	19880	—	30 c.c. 9/7/30	—	30 c.c. 22·7·30	—	—	14	Reacted from 5th and died 9th day after 1 : 3000 injection	—	—	—
	19884	—	30 c.c. 9/7/30	—	30 c.c. 22·7·30	—	30 c.c. 6/8/30	14	Reacted from 14th and died 20th day after 1 : 3000 injection	—	—	—

Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 1, Number 1, 1933.

Rabies in South Africa.
Occurrence and distribution of cases during 1932.

By W. O. NEITZ, B.V.Sc., and A. D. THOMAS, D.V.Sc., Veterinary Research Officers, Onderstepoort.

Du Toit (1929) and Neitz and Marais (1932) have already dealt at some length with the occurrence of rabies in South Africa from 1893 and even earlier known or suspected cases to the end of 1931. These authors have mentioned the peculiarities exhibited by the disease in this country, for instance those associated with its propagation by small wild carnivora (Viverridae), and its apparent failure to become epidemic in domestic carnivora. This naturally has cast some doubt on the identity of the South African virus with that of Europe.

The importance of the disease from the Public Health point of view, and the necessity of paying the closest attention to its spread, or any other development, need no emphasis. Realizing, however, how very little is known of the propagation in nature beyond the fact that Viverrids are carriers, one cannot but experience a certain degree of uneasiness on account of the wide distribution of wild and domestic carnivora roaming uncontrolled all over the country, neither can one ignore the possibility of spread of rabies to the denizens of our national parks and the possible consequences which are almost unthinkable.

Discussion of the cases.

During the year 1932 some fourteen outbreaks of rabies have definitely been diagnosed in the Union. For the sake of convenience the available details of each case are tabulated below (Table I). This number represents an increase in positive cases diagnosed as compared with any of the previous years. It is possible that this increase may be ascribed purely to the fact that the prevalence and danger of rabies are gradually becoming appreciated by the public, so that greater efforts are being made to send material suitable for laboratory examination. Whether it is also due to further spread of the disease amongst natural carriers is impossible to say. The statistics nevertheless give some cause for alarm which is mitigated only by the apparent difficulty with which the disease spreads to other carnivorous animals. The cases recorded are usually traceable to a direct bite by an infected Viverrid.

It is not possible in this short paper to discuss every case at length, but attention should be drawn to a few of the outstanding ones.

Nos. 3 and 8 in Table I are the first two positive cases of rabies demonstrated in the domestic cat in South Africa. The first cat actually bit three persons who fortunately were successfully inoculated with anti-rabic vaccine. There is no precise history as to how these cats became infected, but it is presumed that the disease resulted from the bites of infected meercats. Suspected cases of rabies in the domestic cat had been reported previously, nut no material suitable for diagnosis having ever been submitted, confirmation was impossible.

No. 11 is interesting in that it was possible for the first time to note the incubation period in a dog after natural infection. The symptoms developed in the dog 14 days after a fight with a genet cat (*G. felina*). Furthermore, one of the rabbits (R828) subinoculated from this dog showed an unusually long duration of the disease (11 days) as compared with 3 to 6 days commonly seen in most of the other test rabbits.

A summary of the occurrences of rabies during the year 1932 is given in the appended Table II.

TABLE II.

Year.	Rabies diagnosed in.	Number of Cases in Transvaal.	No. of cases in Orange Free State.	No. of Cases in Cape Province	Total. No. of Cases.
1932...	Human Beings..............	1	2	1	3
	Dog	—	-	1	1
	Ox........................	1	-	1	2
	Domestic cat.	1	1	-	2
	Genetta felina.....	—	—	3	3
	Cynictis penicillata	2	4	-	6
	Suricata suricatta............	—	1	—	1
	TOTAL FOR THE YEAR...	—	—	-	18

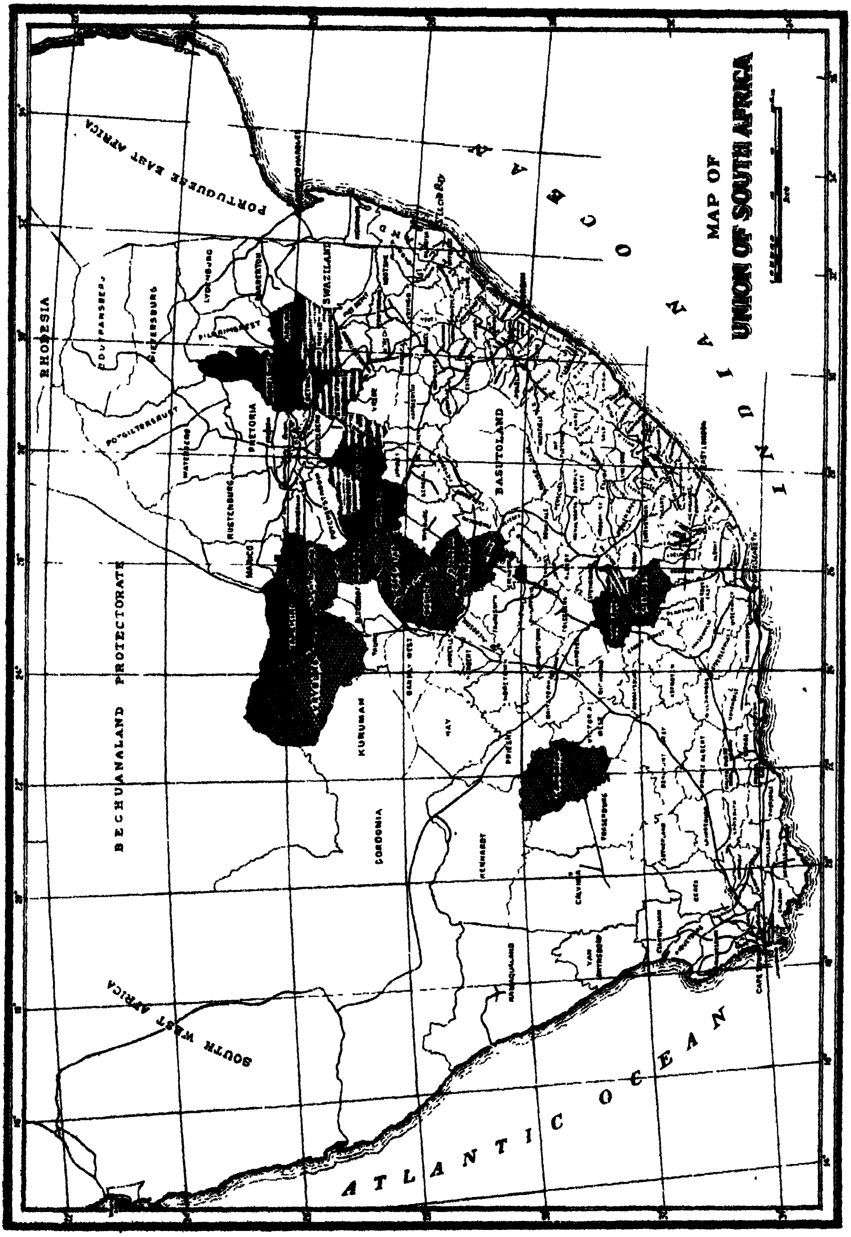
•

DISTRIBUTION OF THE DISEASE.

A map is appended showing the districts in which rabies is known to have occurred. The areas shown should not be taken as indicating the limits of infection today, since our data in this connection are far too scanty. It is more than likely as time goes on that it will be found that in many intervening districts infection is present or has been present for some time. Only by comparing data such as this will it be possible to obtain an idea as to whether the disease is spreading. From the map it will be seen that Mafeking, Cape Province, Kroonstad, Orange Free State, Heilbron, Orange Free State, Middelburg, Transvaal and Carolina, Transvaal (shaded black), are included for the first time as districts in which positive case of rabies have occurred. The other districts indicated by cross-hatching are areas where definite cases of rabies have occurred before, and in those indicated by hatching suspected cases of rabies have been reported.

TABLE I.

Month and Year.	Locality.			Spec. No.	File No.	Origin of material.	Wild animal concerned in original material.	Histo-Patho. Exam. of original material.	Biological Tests.								Remarks.
	Province.	District.	Town or Farm.						Expt. No.	Lab. Animal.	Inocul. from.	Method.	Incub. period in days.	Duration of disease.	Symptoms.	Histo-Path. Examination.	
April, 1932..... Case 1	Tvl.....	Middelburg.......	Uitzicht (farm).........	Sp. 258..	131/676.	*Cynictis penicillata*.....		Negri bodies frequent....	S. 4659	R 666 R 668 R 669	Sp. 258. ,, ,,	Subdural ,, ,,	16 —	1 — —	Appeared dull Did not react —	Positive. Negative .	— — Killed 3½ months later.
May, 1932...... Case 2	Tvl.....	Carolina.........	Van Wyksvlei (farm)...	—	258/283.	European male (44 years)	*Cynictis penicillata*......	-						—	—	—	Diagnosis made by Dept. for Public Health. European developed symptoms 3 weeks after being bitten and, died 6 days later from rabies.
May, 1932...... Case 3	Tvl.....	Wolmaransstad...	Leeuwdoornstad (Townlands)	Sp. 676...	133/2...	Domestic cat........ ..	-	Unsuitable....	S. 4681	R 678 R 681 R 685	Sp. 676 ,, ,,	Subdural ,, ,,	10 12 12	6 5 4	Dumb rabies. Dumb rabies. Dumb rabies.	Positive. Positive. Positive.	Three persons were bitten and no symptoms developed treated with antirabic vaccine.
June, 1932...... Case 4	O.F.S...	Trompsburg......	Trompsburg (Townlands)	Sp. 1765..	141/310.	*Suricata surricatta*......	—	Negri bodies frequent...	S. 4717	R 702 R 703	Sp 1765 ,,	Subdural ,,	17 17	5 3	Dumb rabies. Dumb rabies.	Positive. Positive.	— .
June, 1932...... Case 5	C.P......	Vryburg.........	Farm Boston..........	Sp. 2221G. 2222F.	144/474.	Ox...................	-	Negri bodies frequent .	S. 4732	R 708 R 709	Sp. 22219	Subdural ,,	12 14	1 2	Appeared dull Dumb rabies.	Positive. Positive.	— —
June, 1932...... Case 6	C.P.....	Mafeking........	Farm The Grange......	—	258/283.	Native Male (50 years)..	*Genetta felina*.....	-						-	—	—	Diagnosis made by Dept. for Public Health, confirmed by laboratory examination. Native developed symptoms 3 weeks after being bitten on the upper lip and died 6 days later from rabies.
July, 1932...... Case 7	O.F.S...	Trompsburg......	Trompsburg Townlands.	Sp. 2957G. 2958F	141/310.	*Cynictis penicillata*......	—	Negri bodies fairly frequent	S. 4752	R 722 R 723	Sp. 2957G. ,,	Subdural ,,	14 13	4 4	Dumb rabies. Dumb rabies.	Positive. Positive.	Age of cat 11½ years Showed symptoms of rabies for 7½ days.
July, 1932...... Case 8	O.F.S...	Brandfort........	Farm Saltpan..........	Sp. 3123F. Sp. 3124G.	141/1798	Domestic cat.........	—	Negri bodies frequent....	S. 4754	R 726 R 727	Sp. 3124G. ,,	Subdural ,,	14 14	3 4	Dumb rabies. Dumb rabies.	Positive. Positive.	- —
August, 1932.... Case 9	C.P.....	Mafeking........	Farm The Grange......	Sp. 3293..	144/202.	*Genetta felina*..........	-	Negri bodies frequent . .	-	.	—	--		—	—	--	Biological test not carried out.
Sept., 1932..... Case 10	O.F.S ..	Hoopstad........	Farm Holfontein (Bultfontein)	Sp. 4660G. Sp. 4663F.	141/1634	*Cynictis penicillata*......		Negri bodies present....	S. 4788	R 752 R 753	Sp. 4660G. ,,	Subdural ,,	13 17	2 4	Dumb rabies. Dumb rabies.	Positive. Positive.	— —
Sept., 1932..... Case 11	C.P.....	Vryburg.........	Farm Middlebult.......	Sp. 5713G. Sp. 5714F.	141/82..	Dog..................	*Genetta felina*..........	Negative for Negri bodies	S. 4804	R 804 R 805 R 826 R 827 R 828	Sp. 5713G. ,, R804 & 805 ,, ,,	Subdural ,, ,, ,, ,,	30 13 17 18 16	2 3 6 4 11	Dumb rabies. Dumb rabies. Dumb rabies. Dumb rabies. Dumb rabies.	Negative Negative Positive. Positive. Positive.	The dog showed symptoms of rabies 14 days after having been bitten by Genetta felina.
Oct., 1932...... Case 12	O.F.S ..	Kroonstad..	Wesselsbron (Farm).....	Sp. 6086G. Sp. 6087F.	141/2474	*Cynictis penicillata*......		Negri bodies present.....	S. 4820	R 812 R 813	Sp. 6086G. ,,	Subdural ,,	10 10	3 3	Dumb rabies Dumb rabies.	Positive. Positive.	— —
Dec., 1932...... Case 13	Tvl.....	Wolmaransstad...	Farm Leeuwbos (Leeuwdoornstad)	Sp. 9725G. Sp. 9726F.	139/133.	Ox...................	—	Negri bodies frequent..	S. 4915	R 986 R 969	Sp. 9725G. ,,	Subdural ,,	10 12	5 3	Dumb rabies. Dumb rabies.	Positive. Positive.	— ...
Dec., 1932...... Case 14	O.F.S...	Heilbron.........	Farm Vaalbank........	—	258/283.	European Male (12 years)	*Cynictis penicillata*.....	—	—	—	—	-	-	—	—	—	Diagnosis made by Department for Public Health confirmed by laboratory examination. European developed symptoms 30 days after being bitten and died three days later from rabies.



ACKNOWLEDGEMENTS.

Thanks are due to Mr. F. Boughton for assisting in the operations and in keeping records, to Mr. I. Walker for preparing the map, and to the Department for Public Health for information supplied.

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Section III.

Parasitology.

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Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 1, Number 1, 1933.

The Administration of Anthelminthics to Horses in Bran.

By H. O. MÖNNIG, B.A., Dr.Phil., B.V.Sc., and
I. P. MARAIS, B.Sc. Agric., B.V.Sc., Veterinary Research Officers, Onderstepoort.

The best anthelminthics for horses are Carbon bisulphide (25 c.c. per 1,000 ℔. or more for *Ascaris, Habronema and Gastrophilus*), Carbon tetrachloride (50–100 c.c. per 1.000 ℔. or more for blood-sucking *Strongyles*) and Chenopodium oil (16 c.c. per 1,000 ℔. or more for non-blood-sucking *Strongyles* and *Oxyuris*). These drugs have to be administered in capsule or by stomach tube, although Chenopodium oil can be given as a drench mixed with raw linseed oil. In the case of *Habronema* the stomach should first be washed out with 8–10 litres of a 2 per cent. sodium bicarbonate solution through a stomach tube.

It is obvious, that treatments of this nature can be carried out safely only by a veterinarian and not by the farmer. In South Africa the Government Veterinary Officer is frequently not available for attention to such cases and, while the farmers have not yet realised the value of veterinary assistance, there are relatively few veterinarians in private practice. The difficulty therefore arises, that advice is frequently sought with regard to the treatment of horses for worms but the really useful drugs cannot be prescribed on account of the difficulty of administration.

Roger, Jouveaux and Plateau (1928) described a method of administering these anthelminthics in bran. These authors state, that they have treated a large number of French army horses in this way with great success. The method is briefly as follows :—The horse is placed on sloppy diet for at least 12 hours and then fasted for 36 hours before treatment. Half the dose is mixed with four litres of water in a suitable vessel and, while it is being stirred vigorously, six to seven litres of bran are added until all the liquid is absorbed. The mixture is fed immediately, the first half dose being given in the morning and the second in the same way in the evening.

It was considered desirable to carry out a critical test of this method. The treatment was carried out exactly as described and the worm infection was determined by means of egg-counts on the faeces.

PRELIMINARY TESTS.

1. Horse 20313. Eggs per gram faeces on 26/3/32 : 800 strongyle eggs. Treated with 16 c.c. Chenopodium oil on 4/4/32. Both portions were taken readily. Egg count 21/4/32 : 1,500.

2. Horse 20327. Egg count 26/3/32 : 2,100 strongyle eggs. Treated with 40 c.c. Carbon tetrachloride on 4/4/32. Both portions were taken readily. Egg count 21/4/32 : 230.

3. Horse 20192. Egg count 31/3/32 : 2,200 strongyle eggs. Treated with 50 c.c. carbon tetrachloride on 11/4/32. Both portions were taken readily. Egg count 10/5/32 : 1,200.

4. Horse 19580. Egg count 12/4/32 : 5,650 strongyle eggs. Treated with 16 c.c. Chenopodium oil on 25/4/32. Both portions taken readily. Egg count 10/5/32 : 350.

Discussion. On the whole the results indicated the desirability of further, more accurate investigation.

MORE DETAILED TESTS.

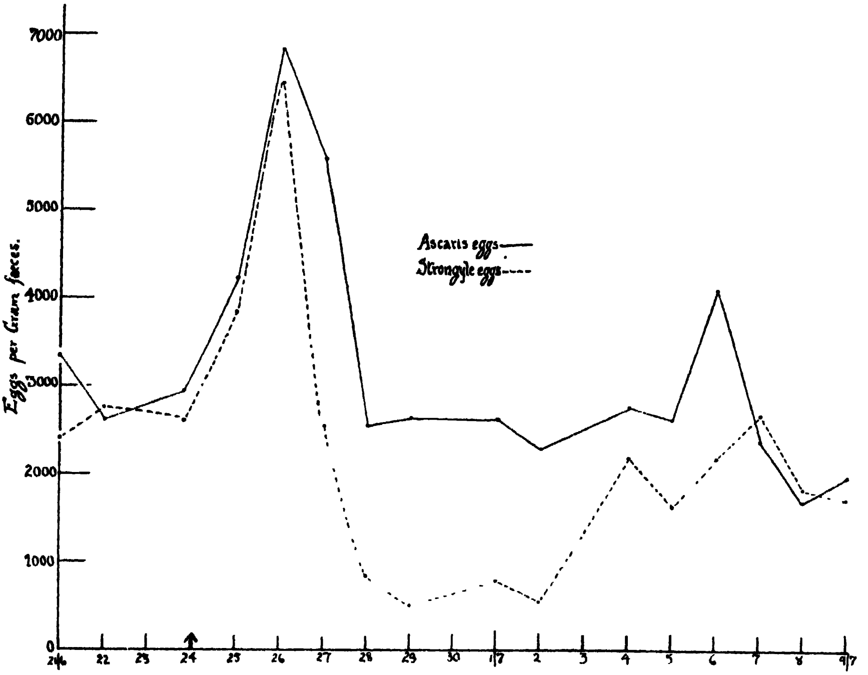
1. Horse 20312. Egg counts on 12-14/5/32 : 6,600, 5,400, 7,350, average 6,450 strongyle eggs per gram faeces. Treated 16/5/32 with 16 c.c. Chenopodium oil. Both portions taken readily. Egg counts on 1-3/6/32 : 10,500. 7,100, 8,650, average 8,750 strongyle eggs per gram faeces.

2. Horse 19177. Egg counts on 12-14/5/32 : 5,000, 3,250, 4,100, average 4,117 strongyle eggs per gram faeces. Treated 16/5/32 with 50 c.c. Carbon tetrachloride. Both portions taken readily. Egg counts on 1-3/6/32 : 9,100, 11,700, 9,350, average 10,050 strongyle eggs per gram faeces.

3. Horse 20369, weight 750 lb. Egg counts on 19-21/5/32 : 6,400, 7,050, 7,100, average 6,850 strongyle eggs and 100, 400, 350, average 283 Ascaris eggs per gram faeces. Treated 24/5/32 with 18 c.c. Carbon bisulphide. Both portions taken readily. Egg count on. 8/6/32 : 11,200 strongyle and 1,800 Ascaris eggs per gram faeces,

4. Horse 20370, weight 700 lb. Egg counts on 19-21/5/32 : 2,250, 3,100, 2,500, average 2,616 strongyle eggs and on 21/5/32 : 50 Ascaris eggs per gram faeces. Treated 24/5/32 with 36 c.c. Tetrachlorethylene. First portion taken readily, but of second half only two-thirds was eaten. Egg count on 8/6/32 : 3,650 strongyle eggs and 200 Ascaris eggs per gram faeces.

It was then decided to treat horse 20369 again and to administer the Carbon bisulphide in one dose. At the same time egg counts were made daily, in order to follow the effect of the drug on the egg-laying activities of the worms. The animal had been stabled for a few weeks and was apparently losing some of its strongyle infection while the ascarids were probably young and beginning to lay. The animal was treated on 24/6/32 with 18 c.c. Carbon bisulphide in the usual quantity of bran mash, which was eaten readily. The results of the egg counts are represented in the following graph :—



Discussion of this case.—The rise in the egg count immediately after treatment may have been due to disintegration of worms killed, but as no ascarids were passed either entire or in fragments and the number of eggs did not decrease subsequently, this rise must be ascribed to the smaller amount of faeces passed on account of the starvation preceding treatment, which would obviously affect the number of eggs per gram. The treatment does not seem to have had any effect on the egg-laying activities of the ascarids, but depressed egg-production of the strongyles up to 8 days after treatment.

Horses 20369 and 20370 were subsequently treated with Carbon bisulphide administered by stomach tube. In the case of 20369 the stomach was first washed out with 8 litres of 2 per cent. sodium bicarbonate, as for the treatment of Habronema. On faecal examination 14 days and 1 month after treatment no Ascaris eggs and only a small number of strongyle eggs were found in both horses.

SUMMARY AND CONCLUSIONS.

The method described by Roger, Jouveaux and Plateau of administering anthelminthics in bran to horses, was found to be quite ineffective against strongyles and ascarids while the administration of Carbon bisulphide by stomach tube was completely effective against Ascaris and fairly effective against strongyles.

LITERATURE.

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Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 1, Number 1, 1933.

The Cause of Nodular Enteritis in Cattle.

By H. O. MÖNNIG, B.A., Dr.Phil., B.V.Sc., Veterinary Research Officer, Onderstepoort.

In 1930 Bontz and Krause examined the worm larvae found in nodules occurring in bovine intestines imported into Germany from North-, Central- and South America, Holland, Australia and Denmark, as well as from cattle in Germany. They discuss the literature on the structure and pathogenic effects of these nodules, mentioning intussusception as one of the results. It is now generally agreed that these nodules are not the cause of the latter condition, which occurs without any nodules being present and is probably due to a change of diet co-operating with an abnormality of the bowel.

These authors give a fairly good description of the larvae, but misinterpret the nature of certain structures and artefacts. They conclude, that the larvae are not those of *Oesophagostomum radiatum* but belong to the genus *Bunostomum*, especially on account of the fact, that there is a large buccal capsule with teeth at its base and that the vulva is situated anteriorly.

In order to settle this question the writer obtained bovine intestines with nodules from the Pretoria abattoir and intestines of sheep which had suffered heavily from *Oesophagostomum columbianum* and had never been in an area where *Bunostomum* occurs. The larvae obtained alive from these nodules were washed in physiological saline, fixed in 70 per cent. alcohol and examined in a mixture of equal parts of 70 per cent. alcohol and lactophenol.

That the larvae from the sheep intestines are those of *O. columbianum* cannot be doubted. Veglia (1923) also describes the larvae from lambs which had been infected experimentally with this species and had been raised free of all other worms and the material from sheep studied in the present case agrees fully with Veglia's description. From the figures it will be quite obvious that the larvae from the bovine nodules are also *Oesophagostomum* larvae, probably *O. radiatum*.

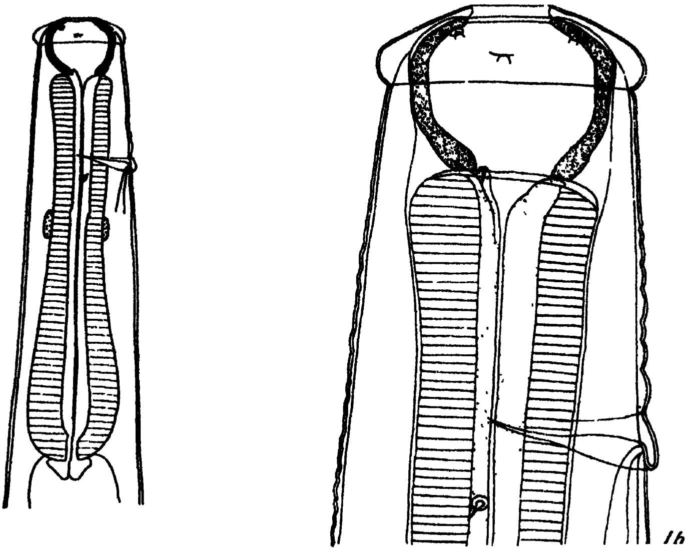

FIG. 1 (*a*) and (*b*).—*Oesophagostomum* larva from intestinal nodule of sheep.

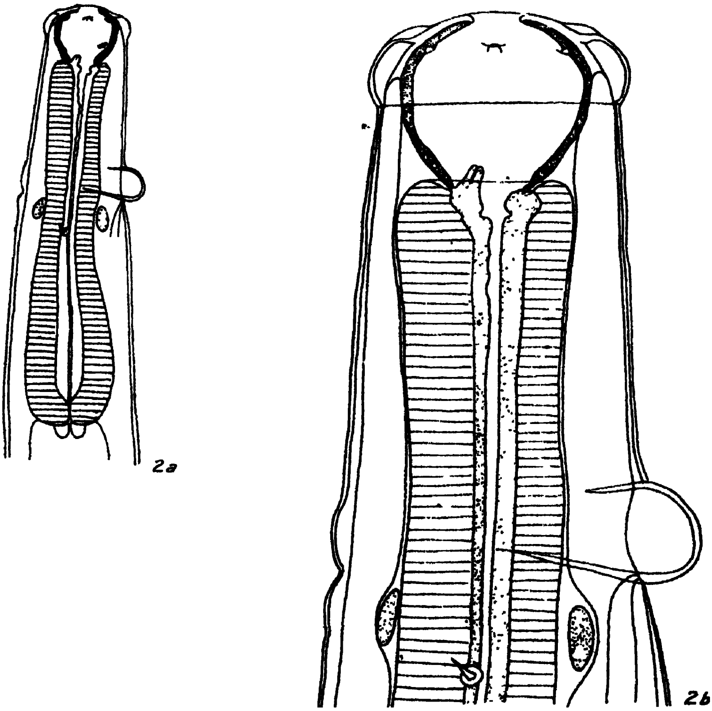

FIG. 2 (*a*) and (*b*).—*Oesophagostomum* larva from intestinal nodule of cattle.

A table of measurements is given below and only a few further remarks are necessary.

The mouth opening is surrounded by two lateral and four sub-median papillae. At the base of the buccal capsule there is a dorsal tooth, larger in the larvae from cattle than in those from sheep. The ventral cervical groove is prominent and characteristic of worms of this genus. Bontz and Krause describe and figure this groove with its cuticular swelling, but state that it is partly due to the prominence of the excretory pore, which opens at this level, and partly an artefact produced in handling the worms.

The female larvae have narrow tails, while the cloacal region of the male larvae is thicker and the tail shorter. The rudiments of the genital organs in both sexes are quite small at this stage and very indistinct. Veglia states, in connection with *O. columbianum*, that the larvae are 1.600–1.700 μ (or 1·6–1·7 mm.) long eight days after infection and the "genital primordium in the female now consisted of eight cells." Bontz and Krause were apparently misled by an artefact in one specimen, which appeared to have a vulvar opening a short distance behind the oesophagus and coiled uterine tubes running forward. Larvae at this stage could in any case not have a vulva and the presence of distinct uterine tubes is not discerned before the development has progressed much further. The "coiled uterine tubes" were possibly the necks of the cervical glands which are very large in these larvae. In the larvae from sheep the glands extend backwards to the end of the second third of the body, while in those from cattle the glands lie diagonally and the posterior one ends usually about one-eighth of the body-length from the posterior extremity.

	Larvae from Sheep.		Larvae from Cattle.		Larvae from Cattle.
	Male.	Female.	Male.	Female.	Bontz and Krause.
Length	1·89—2·33	2·29—2·49	2·52—2·71	2·74—3	1·5
Breadth	0·1	0·1	0·1	0·097—0·1	—
Oesophagus, length	0·3—0·38	0·35—0·37	0·345	0·345—0·37	0·2592
Nerve ring from ant. extremity	0·2	0·2	0·19—0·2	0·2	—
Cervical papillae fr. ant. extremity	0·17	0·17	0·18—0·2	0·2—0·22	—
Excretory pore from anterior extremity	0·15	0·15	0·14—0·18	0·17—0·19	—
Tail length	0·06—0·1	0·11—0·14	0·09—0·1	0·12—0·14	0·1134
Buccal capsule, depth	0·056	0·056—0·06	0·06	0·056—0·06	0·0486

(All measurements in millimetres.)

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VEGLIA, F. (1923). "Preliminary Notes on the Life-History of *Oesophagostomum columbianum*." *9th and 10th Rept. Dr. Vet. Ed. & Res.*, pp. 810–823.

Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 1, Number 1, 1933.

The Chemotherapy of Oesophagostomiasis in Sheep.

By H. O. MÖNNIG, B.A., Dr.Phil., B.V.Sc., Veterinary Research Officer, Onderstepoort.

I. INTRODUCTION AND GENERAL DISCUSSION.

Before the introduction of the Government Wireworm Remedy as a chemotherapeutic agent for the control of Haemonchosis in sheep, the position with respect to this parasite had become so acute in many parts of South Africa, that sheep farming was a rather hopeless venture, as a large percentage of the lamb crop was lost annually. It was soon found, that regular dosing at intervals of about three weeks, as indicated by the life-history of the parasite, not only controlled the wireworm in a very effective manner, but also brought about a marked improvement with respect to other worm parasites. The sheep, having obtained relief from the most serious of its internal parasites, was able to combat the rest on account of its increased vigour and the improvement in its general condition. It is a well known fact, that those farmers who have practised such regular dosing, are not greatly concerned about worms in sheep any longer.

Unfortunately the high price of wool some years ago brought about a great increase of sheep farming, in many cases by farmers who had little knowledge of worm diseases and took little trouble to acquire this knowledge. At the same time the way for further trouble was being paved by much overstocking. The result is, that the nodular worm, *Oesophagostomum columbianum*, has been able to develop in sheep that were not treated for wireworm and, aided by overstocked pastures, it has become so prevalent in many areas that, even though the sheep may now be regularly treated for wireworms in some cases, the infection with nodular worm is too severe for the sheep to overcome. The nodular worm is at present rapidly becoming as great a menace to the sheep industry as the wireworm was formerly.

Investigations in connection with the chemotherapy of this parasitic disease were started by the writer in 1922, but the work was interrupted and restarted in 1927, since which time a large number of tests have been made. Although no finality has been reached, it is considered desirable to publish what information has been obtained in the course of the work, as it may give other investigators some useful information, especially with regard to the exact nature of the problem.

In this paper treatment by administration of drugs per os only will be dealt with.

It was realised from the beginning, that the difficulty would be to get the drug to the worms. Several methods were tried to attain this object and it will be seen that there are several quite useful drugs, but that it appears to be a waste of time and material to proceed with such tests until the fundamental problem has been solved. This is the physiology of deglutition in the sheep. In other words, a method of drug administration will first have to be found, which will cause the sheep to swallow the material directly to the abomasum.

It became quite clear during the course of the work that, when a suitable drug is swallowed into the abomasum, it can effectively deal with the nodular worm but, when it is swallowed into the rumen and becomes diluted in the large quantity of ingesta, eventually being passed on in small quantities with food, the result will be nil.

Since ordinary dosing will cause the drug to be swallowed either into the rumen or the abomasum, a drug may be tested on several sheep, which may all swallow it to the rumen. The result will be negative and the drug may be discarded as useless, although it may be quite effective when swallowed into the abomasum. It is therefore clear, that such testing is misleading and a waste of energy and material.

Several authors have published the results of their investigations with regard to the administrations of drugs into the abomasum. Their work was carried out chiefly in connection with fluids. For the treatment of nodular worm in sheep a fluid drug is not as suitable as a powder, mainly on account of the factor of absorption. It will be seen, that fairly insoluble drugs seem to offer the greatest possibility of success. The problem thus presents itself in the first instance as a question of administering a powder into the abomasum and investigations with this object in view have been in progress here for some time.

This question of dosing into the abomasum is the most important problem to be solved in connection with the treatment of sheep for gastro-intestinal worms. It would appear from the work of Green (1918) and Veglia (1918) on the chemotherapy of haemonchosis and from the wide experience gained in the treatment of sheep for this disease, that in this case it makes no great difference whether the drug employed reaches the abomasum in a concentrated form or in small quantities from the rumen and reticulum over a prolonged period. Green (1918) states, in connection with the Government Wireworm Remedy, " It seems probable that whenever the dose goes *wholly* into the rumen the concentration in the abomasum rarely exceeds ·001 - ·002 per cent As_2O_3 at any time. That so low a concentration, even after prolonged action, is effective in destroying the wireworms is not absolutely certain, but is highly probable . . . Veglia's observations, and the uniformly successful results of dosing on the large scale in the field, suggest that the wireworms are destroyed irrespective of the path taken by the dose, and are, therefore, susceptible to slow intoxication by very low concentration of arsenic." This remedy, of course, contains, besides arsenic, also a large proportion of copper sulphate, which was, however, not considered in the paper referred to.

In spite of these findings, it does appear desirable to dose such drugs also into the abomasum, as that would probably allow a decrease to be effected in the dose rate, resulting in greater safety. With the present state of affairs the dosage must be sufficiently high to be effective in those cases in which the drug is swallowed to the rumen, while it must be as small as possible to be safe.

It is further quite obvious, that the solution of this problem will do away with some of the difficulties experienced in the treatment of infections with small strongyles (*Trichostrongylus*, *Ostertagia*, etc.) and tapeworms in sheep.

The question of preliminary starvation necessarily crops up and will be dealt with briefly. There are two aspects to be considered: the necessity of starvation of sheep in a general way and the role of starvation in the technique of administering drugs into the abomasum. With regard to the first point, the writer has several years ago discussed the theoretical aspect of the matter and tested dosing without starvation for wireworms on a large scale (Mönnig, 1929 *a* and *b*), with the result that this method is now generally recommended and practised with great success. The method has been criticised, especially by le Roux (1932), but the occurrences on which he bases his criticism were complicated by other factors and do not allow a definite decision on the question of starvation. Since that time several carefully controlled tests have been made, for instance, regular three weekly dosing without starvation of 90 young sheep running on infected pasture for a period of almost a year, and the results have proved the method to be satisfactory. Even though it may eventually be shown, that such dosing is not quite as effective as with preliminary starvation, which the writer doubts, it would seem more rational to remove a slightly smaller number of the worms and avoid starvation with its effect on the sheep and other incidental disadvantages, than to remove a few more worms and have all the disadvantages, especially since regular dosing is the only satisfactory method of treatment and with such a procedure 100 per cent. efficacy is not necessary.

The importance of this question becomes evident when the second point is considered, namely the role of starvation in the technique of administering drugs into the abomasum. Whether starvation will assist in inducing sheep to swallow into the abomasum is not definitely settled, but it does not seem to be of any consequence. Theoretically, starvation of sheep is apparently believed to cause a decrease of the abomasal contents or even to empty this organ of ingesta. The facts of this matter will be discussed again later. Supposing, however, that this is true, it does not appear to be desirable to administer an irritant or corrosive drug into an empty abomasum and hence starvation would seems to be contraindicated in such a method of dosing, especially as we know that the abomasal contents will not dilute the drug to such an extent that it becomes ineffective against wireworms. In the case of treatment for nodular worm, the drug, after being swallowed into the abomasum must be passed on as rapidly as possible to the colon. Starvation tends to cause stagnation of the stomach contents and is, therefore, contraindicated, as it would retard the passage of the drug on its way to the nodular worm, even if the sheep be fed immediately after dosing.

The facts with regard to the fate of ingesta in the different compartments of the ruminant stomach, as affected by starvation, have been studied in th investigations of Green and Veglia referred to above. Green states: "According to Veglia's observations rumination was marked after a feed, but became more and more remittent as the starvation period lengthened, until after seventeen hours it practically ceased. At the same time the semi-solid contents of the rumen were not reduced to the extent which might have been expected, but maintained a minimum average bulk, and in some cases remained as high as 4·5 Kilos after seventeen to twenty-four hours starvation. The contents of the rumen and reticulum in sheep killed two to four hours after feeding were in general not much greater than in those killed after a day's starvation, and individual variation smothered all clear differences. The contents of the abomasum varied more, and, although in some cases the bulk after long starvation was suggested as lower than after short starvation, the differences were by no means so marked as might have been expected, but were, on the whole of an arbitrary character. The extreme range in the abomasal contents was 25 c.c. to 940 c.c. for seventeen sheep killed two to four hours after feeding, and 55 c.c. to 360 c.c. for twenty-seven sheep killed after seventeen to twenty-four hours starvation." The writer can fully endorse these statements from his experience. It seems as if the ruminant stomach normally retains a fairly large minimum bulk even after prolonged starvation and that the content of the abomasum is very variable as stated by Green. Starvation thus causes stagnation and not an emptying of the stomach. If these facts are correct, and they must, if exceptional, produce a very large proportion of exceptional cases, starvation is certainly contraindicated when the drug has to be passed on rapidly from the abomasum, as in the treatment of oesophagostomiasis.

II. EXPERIMENTAL WORK.

The nature of the problem is now clear and it will be obvious that the tests of drugs to be recorded here are of a preliminary nature only. It will therefore be attempted to summarise the results as briefly as possible.

The technique employed throughout the tests is that described by the writer (1931). In short, the sheep are infected with larvae of *Oesophagostomum columbianum* and when the worms are adult the nature and degree of infection are determined by faeces cultures and egg counts. In the tests recorded below egg counts were, as a rule, not made as the tests were preliminary "feelers." The selected sheep are treated and the faeces examined daily for at least a week or until no more worms are passed. Faeces cultures and egg counts are again made a fortnight after treatment to determine the remaining infection.

Since purgation mechanically removes some nodular worms from sheep, tests in which the drugs caused purgation are not taken into account as the effects cannot be ascribed directly to the drug. Any other course would be misleading, since the drug cannot be depended upon to cause purgation regularly and removal of the worms by purgation is neither dependable nor desirable in many cases.

(*a*) Soluble Drugs.

Little need be said under this heading. It is well known that relatively soluble drugs which have been tested so far have had no effect. Tests made by the writer with such drugs gave the same result. The following table shows the results obtained with a few of the drugs selected from the list of relatively soluble and fluid drugs tested :—

Table I.

Drug.	Dose in Gm.	No. of Sheep.	Effect.	Remarks.
Aluminium chloride.......	2	2	—	No toxic effects in any of these cases.
Protargol................	1	2	—	
Pyrethrum...............	2·75	7	—	
Thymol..................	4	1	—	
β-Naphthol..............	1	1	Slight.	
Sod. cacodylate...........	0·5 and 1	2	—	
Benzol sulphochloride.....	1 and 2 c.c.	2	—	
Naphthalene tetrachloride..	0·5 and 1 gm.	2	—	
Kamala..................	3	2	—	
Nicotine salicylate........	0·25	1	—	
Nicotine tartrate.........	0·5	1	—	

In this and the following tables the effect is given as "slight" when a small number of the worms present were passed, "fair" when about half or more were passed and "good" when all or almost all were passed.

(*b*) SOLUBLE AND OTHER DRUGS WITH ASTRINGENT AND LAXATIVE.

The idea is to reduce absorption by means of an astringent and to promote rapid passage to the colon by means of a laxative. It is not an easy matter to purge sheep, at least not without very variable results. Consequently a laxative as indicated here will usually not cause purgation ; at most the faeces will become soft. (The writer has had the most uniform results in purging sheep by the administration of 100 gm. magnesium sulphate and 30 gm. sodium chloride in 500 c.c. water by stomach tube into the rumen).

TABLE II.

DRUGS GIVEN WITH 1 GM. POTASSIUM ALUM.

Drug.	Dose in Gm.	No. of Sheep.	Effect.	Remarks.
Thymol	1	1	—	No toxic effects in any of these cases.
Picric acid	0·5	1	—	
Atoxyl	0·5	1		
Tartaric emetic	0·5	1	—	
Copper arsenite	0·5	1	—	
Mercurochrome	0·5	1	—	
Lead arsenate	0·25	1	—	
Government Wireworm Remedy	0·5	1	—	

TABLE III.

DRUGS GIVEN WITH 1 GM. SODIUM ALUM.

Drug.	Dose in Gm.	No. of Sheep.	Effect.	Remarks.
Ammon. sulphate	1	1	—	No toxic effects in any of these cases.
Gentian violet	1	1	—	
Trypan blue	1	1	—	
Potassium iodide	1	1	Slight.	
Santonin	1	1	Slight.	
Pot. ferrocyanide	1	1	—	
Pot. thiocyanate	1	1		
Antimony trioxide	1	1	—	
Calomel	0·5	1	—	
Mercuric sulphate	0·5	1	Fair.	

TABLE IV.

DRUGS GIVEN WITH 1 GM. TANNIC ACID.

Drug.	Dose in Gm.	No. of Sheep.	Effect.	Remarks.
Ammon. sulphate	1	1	—	—
Gentian violet	1	1	—	—
Trypan blue	1	1	Slight.	—
Potassium iodide	1	1	Slight.	—
Santonin	1	1	Slight.	—
Pot. ferrocyanide	1	1	—	—
Pot. thiocyanate	1	1	—	—
Antimony trioxide	1	1	Slight.	—
Calomel	0·5	1	—	Died of gastroenteritis.
Mercuric sulphate	0·5	1	Fair.	—

TABLE V.

DRUGS GIVEN WITH 0·5 GM. CALOMEL.

Drug.	Dose in Gm.	No. of Sheep.	Effect.	Remarks.
β-Naphthol	1	1	—	No toxic effects in any of these cases.
Copper tartrate	1	2	Slight to 0.	
Arsenious sulphide	1	7	Good (1), Fair (3), Nil (3).	
Picric acid	0·5	2	—	
Trypan blue	1	2	—	
Potassium iodide	1	1	-	
Sulphur	2 & 5	2	—	
Barium arsenate	1	2	Fair.	
Bismuth carbonate	2	2	—	

TABLE VI.

DRUGS GIVEN WITH 0·5 GM. CALOMEL+1 GM. SODIUM ALUM.

Drug.	Dose in Gm.	No. of Sheep.	Effect.	Remarks.
Trypan blue	1	3	Slight (1), Nil (2).	No toxic effects in any of these cases.
Tartar emetic	0·5 & 1	3	Slight (2), Nil (1).	
Santonin	1 & 2	3	Slight (2), Nil (1).	
Calomel and alum alone	0·5 & 1	3	Slight (2), Nil (1).	

Discussion.—On the whole this line of attack does not appear very promising. The drugs that gave the best results were relatively insoluble, like Mercuric sulphate and Barium arsenate, which also produced similar results when given alone.

(c) RELATIVELY INSOLUBLE DRUGS.

The feeding habits of the worm will naturally play an important part in the chemotherapy against it. Observations have been made by the writer on the feeding habits and the question is being further investigated. It seems as if the worm ejects through its mouth a digestive fluid, probably secreted by the oesophageal glands. The attack is probably directed mainly against the mucous membrane of the colon, although the worms are never found to adhere to it. The pre-digested material is then ingested by the parasite. This seems to be the method of feeding of this worm and would be comparable to the feeding habits of other worms like the small strongyles of the horse, according to Wetzel (1930) and *Proleptus obtusus* according to Schuurmans-Stekhoven and Botman (1932), although these worms attach themselves to the mucous membrane.

Apart therefore from the possibility of getting relatively insoluble chemicals to reach the colon, the feeding habits of the worm would apparently enable it to swallow small particles or solutions of drugs present in the contents of the colon and the drugs may then act on the worm. This expectation appears to have been realised in the tests with relatively insoluble drugs and so far the best results have been obtained with this method of treatment. The drugs used singly are given in the following table:—

TABLE VII.

RELATIVELY INSOLUBLE DRUGS.

Drug.	Dose in Gm.	No. of Sheep.	Effect.	Remarks.
Antimony arsenate	0·5 & 1	6	Slight (1), nil (5)	—
,, ,,	2	1	Fair	—
,, oxalate	1 & 2	3	—	—
,, ,,	4	1	—	Died of gastroenteritis.
,, trisulphide	1	1	—	—
,, pentasulphide	1	1	Slight	—
Arsenic bisulphide	1	1	Slight	—
,, trisulphide	0·25	5	Good (5)	Variable action is probably due to direction taken at deglutition.
,, ,,	0·3	6	Good (2), Nil (4)	
,, ,,	0·6	3	Fair (1), nil (2)	
,, ,,	1	15	Good (10), nil (5)	
,, ,,	1·3	1	Good	—
,, pentoxide	0·5	3	Good (2)	Died of poisoning.
Barium arsenate	1	7	Good (1), slight (3)	2 died of worms.
,, carbonate	1	3	Slight (1), nil (2)	
,, ,,	2	2	—	
,, chromate	1 & 2	4	—	—
Bismuth carbonate	1	2	Slight (1), nil (1)	—
,, ,,	2	2	Fair (2)	—
,, ,,	4	2	—	—
,, subgallate	1 & 3	2	—	—
,, subnitrate	1	3	Slight (2), nil (1)	—
,, trisulphide	1 & 2	2	—	—
Calomel	0·5	4	—	—
Calcium citrate	1	1	—	—
,, fluoride	1	3	Slight (2), nil (1)	—
,, ,,	2	2	—	—
,, oxalate	1, 2 & 4	4	—	—
Copper arsenate	0·5	2	Slight (1), nil (1)	—
,, ,,	1	2	—	—
,, ,,	2	1	—	—
,, arsenite	0·5	3	Fair (2), nil (1)	—
,, ,,	1	3	Slight (1)	2 died of worms.
,, carbonate	0·5	1	Good	—
,, ,,	1	2	—	—
,, ,,	2	2	Fair (1), nil (1)	—
,, oxide (CuO)	0·75	1	—	—
,, sulphide (Cu_2S)	0·5, 1, 1·5	3	—	—
,, tartrate	1	5	Good (1), nil (4)	—
,, thiocyanate	1	4	—	—

TABLE No. VII—(*continued*).

Drug.	Dose in Gm.	No. of Sheep.	Effect.	Remarks.
Ferric acetate	1	2	Slight (1), nil (1)	—
Hexachlorethane	2, 4, 6, 8	4	—	—
Lead sulphide	1 & 2	3	—	—
„ tartrate	1 & 2	4	Slight (1), nil (3)	—
Mercuric arsenate	0·5 & 1	3	—	—
„ oxide (yellow)	0·5	1	Good	Died of poisoning.
	1	2	Good	1 died of poisoning.
„ oxide (red)	1	2	Fair	1 died of poisoning.
„ sulphide (red)	0·5 & 1	3	Slight (1), nil (2)	—
„ sulphide (black)	0·5 & 1	2	—	—
„ thiocyanate	0·5 & 1	2	—	Both died of worms.
Mercurous iodide	1	2	Good	Died of poisoning.
„ sulphate	0·5	2	Good (1), fair (1)	Died of poisoning.
Metachloral	0·5, 1, 2	3	—	—
Naphthalene	1	1	Slight	—
Phosphorus (red)	0·5	1	Fair	Died of poisoning.
Salol	0·5	2	—	—
„	1	6	Slight (2), nil (4)	—
Sodium fluoride	1 & 2	2	—	—
„ fluosilicate	1	1	—	—
„ „	2	2	Good (1), nil (1)	—
„ „	3	1	Good	—
„ „	4	1	Good	Died of " debility."
Tetrachlorethylene	4 c.c.	2	Slight (1), nil (1)	—
„	5 c.c.	3	Fair (1), nil (3)	—
„	10 c.c.	3	Fair (2), nil (1)	—
Thymol biniodide	0·5	1	—	—
„	1	5	Fair (1), nil (4)	—

Discussion.—It is fairly obvious from these results, that there is a factor which causes very unexpected variations in the effects of the drugs. For instance, the very first drug tried against this parasite was Arsenious sulphide (As_2S_3). Two sheep were dosed with 0·25 gm. each and both passed all their nodular worms. Since that time the drug has sometimes acted in a similar way and in other cases not at all, even in 1 gm. doses. Fifteen sheep received 1 gm. and of these ten passed all the worms while five passed none. Had the drug been tested on the latter five only it may have been discarded as useless. Most drugs in the above list were tested on less than five sheep. One hesitates to draw any conclusion from the negative results obtained with many of them.

The most promising drugs under these conditions seem to be Arsenious sulphide and Sodium fluosilicate, while copper and mercury salts may prove useful, but the latter are rather toxic. The mercury salts were expected to give good results, since they would be excreted in the colon if they became absorbed.

On the whole, however, one must conclude that, as stated before, the result may be misleading and quite useful drugs may not have shown up.

" Debility " as used in these tables means the general exhaustion caused by the nodular worm.

The following combinations of drugs were tested:—

TABLE VIII.

Drug.	Dose in Gm.	No. of Sheep.	Effect.	Remarks.
As_2S_3 + Barium arsenate....	0·5 + 0·5....	3	Slight (1), nil (2)....	—
,, + Bismuth carbonate..	2 + 1........	4	Fair (1), nil (3).....	—
,, + ,, ,, ..	4 + 0·5......	2	—	—
,, + ,, subnitrate.	0·5 + 1......	3	—	—
,, + Copper tartrate.....	0·5 + 0·5....	2	—	—
,, + ,, ,, ...	1 + 1........	5	Good (2), nil (3)....	—
,, + Kamala............	1 + 1........	1	—	—
,, + Mercurous iodide....	0·5 + 0·25...	2	—	—
,, + ,, sulphate.	0·75 + 0·25..	2	Good (1), fair (1).....	—
,, + Mercuric sulphide (bl.)	0·5 + 0·5....	2	—	—
,, + β-Naphthol.........	0·5 + 1......	2	—	—
,, + Picric acid..........	0·5 + 0·5....	2	—	—
,, + Potassium iodide....	0·5 + 1......	2	Fair...............	—
,, + Sodium fluosilicate..	1 + 2........	4	Good (2), nil (2)....	—
,, + Trypan blue........	0·5 + 1......	2	—	—
,, + Copper tartrate + Mercurous sulphate.	1 + 1 + 0·25..	2	Slight.............	—
,, + Copper tartrate + Calomel...........	0·5 + 1 + 0·5.	2	—	—
,, + Calomel + Mercurous sulphate...........	0·5 + 0·5 + 0·25	3	Slight (2), nil (1)....	—
,, + Calomel + Potassium iodide + Tannic acid.	0·5 + 0·5 + 1 + 1	2	Good (1), nil (1)....	—
Bismuth carbonate + Barium arsenate................	2 + 0·5......	2	—	—
Copper tartrate + Mercurous sulphate................	0·75 + 0·25...	1	Good (1), nil (1)....	—
Copper tartrate + Barium arsenate................	1 + 1	2	Good (1), fair (1).....	—
Mercuric oxide (red) + Barium arsenate............	0·25 + 0·75..	2	Good..............	1 died of "debility."
Sodium fluosilicate + Mercuric oxide (red).........	2 + 0·15.....	2	Fair (1), slight (1)...	—
Sodium fluosilicate + Santonin....................	2 + 2........	4	Good..............	—

Discussion.—The same remarks that were made with reference to Table VII could be made here. The impression was obtained that arsenious sulphide, in combination with some metallic salts, tends to form sulphides of these metals and that the efficacy of both components is reduced.

SUMMARY.

(1) The problem of chemotherapy in oesophagostomiasis cannot be solved satisfactorily until a method of dosing sheep into the abomasum has been found. With ordinary dosing useful drugs may be swallowed into the rumen and will then not act.

(2) The results obtained with 72 different drugs and various combinations of these, tested on 308 sheep, are tabulated and briefly discussed.

(3) The best results were obtained with relatively insoluble drugs, which are most likely to reach the colon and which may apparently be ingested by the parasites.

(4) The most promising drugs appear to be arsenious sulphide and sodium fluosilicate, while certain compounds of copper and mercury may be useful.

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Wild Antelopes as carriers of Nematode Parasites of Domestic Ruminants—Part III.

By H. O. MÖNNIG, B.A., Dr.Phil., B.V.Sc., Veterinary Research Officer, Onderstepoort.

I. IMPALA (*AEPYCEROS MELAMPUS.*)

THE material studied was collected by Dr. A. D. Thomas and Mr. W. O. Neitz of this Institute in and near the Kruger National Park in the Northern Transvaal, on the 23rd-25th June, 1932. It contained the following:

From the abomasum one *Haemonchus* female, *Cooperia hungi*, *Trichostrongylus thomasi** and *Bigalkea sabie.**

From the jejunum—*Cooperia hamiltoni**, *Impalaia tuberculata* and *I. nudicollis.*

From the colon— *Oesophagostomum columbianum.*

Material of the lungs contained very slender nematodes, which could not be dissected out satisfactorily from the fixed tissues. Apparently similar nematodes were present in the lungs of the blue wildebeest (*Gorgon taurinus*). At a later date fresh lungs of an impala were obtained from the same locality, forwarded in chloroform vapour, and sufficient worm material was obtained to allow a definite identification to be made. The worms proved to belong to a new genus and were described as *Pneumostrongylus calcaratus.*

TRICHOSTRONGYLUS THOMASI, MÖNNIG, 1932.*

Rather small, delicate worms, more or less straight. The cuticle shows fine transverse striations and, in the female, it forms irregular thickenings in the region of the vulva. The excretory pore lies, as is commonly the case in *Trichostrongylus*, in a depression 0·13 to 0·18 mm. from the anterior extremity. The head is about 0·011 mm. wide and the mouth opening is followed directly by the oesophagus, which measures 0·82 to 0·92 mm. in length and is slightly club-shaped. The nerve-ring surrounds the oesophagus 0·68 to 0·71 mm. from the anterior extremity of the body.

The *Males* are 4·19 to 5·3 mm. long and 0·094 to 0·108 mm. wide across the prebursal papillae. The bursa is well developed with large lateral and a small dorsal lobe (Fig. 1). The latero-ventral is the thickest ray, it is widely divergent from the small ventro-ventral and ends in a slender prolongation which curves forwards. The antero-lateral also narrows down suddenly to form a finger-like extremity. The postero-lateral is long, practically reaching the bursal margin. The externo-dorsals are thick at their bases and each has a knob-like swelling near the end with a thin tip. The dorsal bifurcates distally but the branches are not digitate. The spicules are equal and similar, 0·229 to 0·236 mm. long and brown in colour. They are slightly curved and bear a number of ridges (Fig. 2), but have no hook near the end. Around the middle third each spicule bears two small triangular alae, which are not shown in the figure and can only be seen when the spicules have been dissected out. The gubernaculum is 0·13 mm. long, pointed anteriorly, split in the middle and alate posteriorly (Fig. 3).

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* **Short descriptions of the new species have been published in the *Jl. S.A. Vet. Med. Assn.*, Vol. 3, No. 4, pp. 171–175, 1932.**

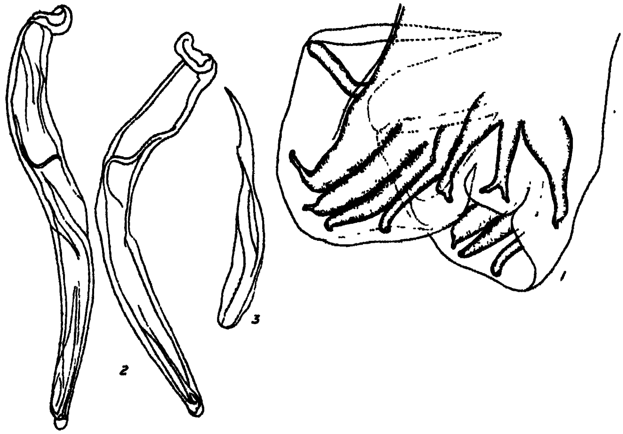


Fig. 1. Trichostrongylus thomasi....... Male bursa.
Fig. 2. „ „ .... Spicules.
Fig. 3. „ „ .... Gubernaculum.

The *females* are 4·2 to 5·6 mm. long and 0·082 mm. wide across the vulva. The tail is 0·07 to 0·11 mm. long and pointed. The vulva is a longitudinal crescent-shaped slit, surrounded by irregular cuticular thickenings and situated 0·92 to 1·19 mm. from the posterior extremity. The ovejectors are well developed, measuring each 0·21 to 0·26 mm. in length, the posterior being usually longer than the anterior. The eggs in the ovejectors measure 0·071 to 0·078 by 0·037 mm.

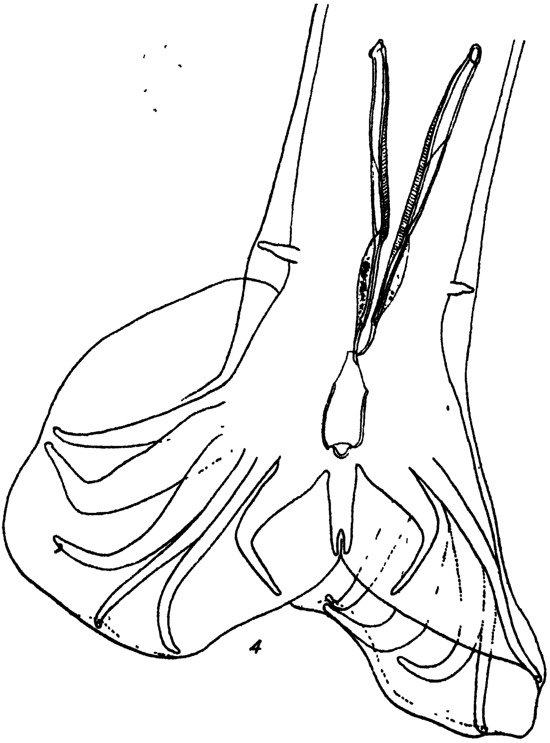
Host—Impala (*Aepyceros melampus*).
Location: abomasum.
Locality: Kruger National Park.
Types in Onderstepoort Helminthological Collection, No. 2466.

I have much pleasure in dedicating this species to my colleague, Dr. A. D. Thomas.

BIGALKEA SABIE, MÖNNIG, 1932.

Delicate, slender worms not coiled. The cuticle bears about 32 longitudinal striations beginning 0·8 to 0·9 mm. behind the anterior extremity which bears transverse striations. The head is simple, 0·034 to 0·04 mm. wide, and not swollen. Cervical papillae are present, triangular in shape and situated 0·35 to 0·42 mm. from the anterior extremity. The excretory pore opens 0·31 to 0·38 mm. from the anterior extremity. The oesophagus is slightly club-shaped, 0·69 to 0·8 mm. long in the males and 0·75 to 0·86 mm. in the females and is surrounded by the nerve-ring 0·29 to 0·35 mm. from the anterior end of the body.

The *males* are 8·19 to 8·82 mm. long and 0·1 to 0·12 mm. broad in front of the bursa. The latter has large lateral lobes, but is devoid of a dorsal lobe (Fig. 4). The latero-ventral ray is larger than the ventro-ventral and slightly divergent, but curves forward again distally. The antero-lateral diverges forwards from the medio-lateral which is straight, while the postero-lateral diverges towards the dorsal side. The externo-dorsal arises from the base of the dorsal stem and are bent sharply inwards at the middle. The dorsal bifurcates distally to form two short, parallel branches, but it is difficult to see clearly where the bifurcation begins. There are two equal, alate spicules 0·16 to 0·176 mm. long. They are partly membranous in the distal half and each ends in a slightly hooked point (Fig. 4). In this respect the spicules of *B. albifrontis* have not been quite correctly described, as they also are partly membranous in the distal half, while they end in three points each. The gubernaculum is broad and measures about 0·07 mm. in length.



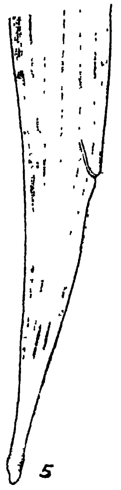


Fig. 4. Bigalkea sabie.................. Bursa and spicules.
Fig. 5. „ .... Tail of female.

The *females* are 11·07 to 15·7 mm. long and 0·124 mm. wide across the vulva. The latter is usually surrounded by lateral cuticular thickenings which bear transverse striations, but there is no flap. The tail is 0·165 to 0·2 mm. long, acute, with a blunt tip bearing a small number of transverse striations frequently on a slight swelling as shown in Fig. 5. The vulva is situated 1·97 to 2·3 mm. from the posterior extremity. The ovejectors are each 0·15 to 0·17 mm. long and the eggs contained in them measure 0·067 by 0·041 mm.

Host—Impala (*Aepyceros melampus*).

Location : abomasum.

Locality : Kruger National Park.

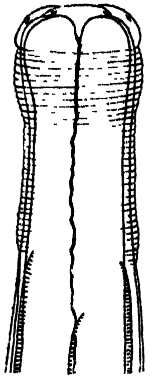
Types in Onderstepoort Helminthological Collection, No. 2465.

This worm is very closely related to the other species of this genus, *B. albifrontis*. It can be distinguished especially by the shape of the spicules, absence of a dorsal lobe in the male bursa, the divergence of the postero-lateral from the medio-lateral rays, the sharp bend of the externo-dorsal, the long stem of the dorsal ray and the appearance of the tail in the female.

COOPERIA HAMILTONI, MÖNNIG, 1932.*

This species, also taken from the Impala, is dedicated to Capt. Stevenson-Hamilton, Curator of the Kruger National Park, who shot the buck and who assisted greatly in making further work along these lines possible.

The worms are coiled anteriorly, as is usual with species of *Cooperia*. The mouth is surrounded by four small lips and two lateral and four submedian small papillae. The lips are followed by transversely striated cuticle reaching up to 0·09 to 0·1 mm. from the anterior extremity, where the longitudinal straitions begin. These are about 12 in number and have the usual comb-like appearance. The transversely striated region is swollen anteriorly, forming together with the lips, a "head," which is 0·045 to 0·049 mm. wide and 0·037 to 0·048 mm. long (Fig. 6). The excretory pore is situated 0·25 to 0·27 mm. from the anterior extremity. The oesophagus is 0·49 to 0·6 mm. long and is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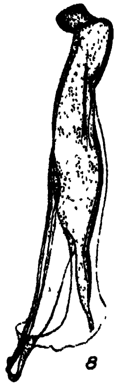
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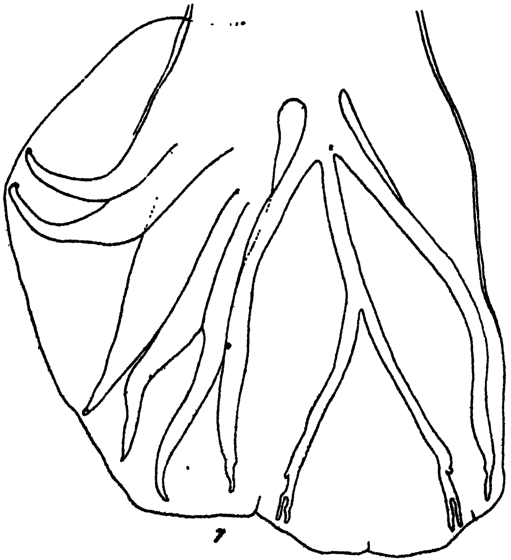
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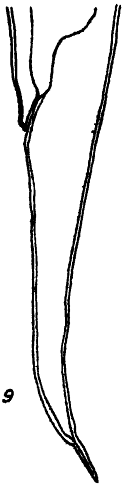
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Fig. 6. Cooperia hamiltoni............. Anterior end.
Fig. 7. " .... Male bursa.
Fig. 8. " .... Right spicule, median view.
Fig. 9. " .... Tail of female.

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*This species would have to be placed in the genus *Cooperioides* Daubney, 1933 (Parasitol. 25 (2): 234) as *Cooperioides Hamiltoni* (Mönnig 1932).

surrounded by the nerve-ring at a distance of about 0·28 mm. from the anterior end of the body.

The *males* are 5·67 to 6·4 mm. long and 0·12 to 0·14 mm. wide in front of the bursa. Small prebursal papillae are present. The bursa is well developed, with lateral and dorsal lobes (Fig. 7). The ventral rays are not far apart and run more or less parallel. The antero-lateral is thick, straight and tapers out to a point, the medio- and postero-laterals are also long and pointed and only slightly divergent. The externo-dorsal arises near to the base of the dorsal ray and is long and slender, lying close to the postero-lateral and ending near the bursal margin. The dorsal ray is long and bifurcates at its middle, each branch being slender and digitate at its extremity near the margin of the bursa.

The spicules are equal and similar, 0·2 to 0·23 mm. long and brown in colour. The body, which is simple, bears a large prominent dorsal spur, which supports a large cuticular expansion, while the distal half of the body itself bears a similar expansion (Fig. 8).

The *females* are 7·2 to 8·2 mm. long and 0·14 to 0·15 mm. wide across the vulva. The tail has a mucronate tip (Fig. 9) and is 0·16 to 0·2 mm. long, the tip measuring 0·19 to 0·034 mm. The vulva is situated 1·32 to 1·6 mm. from the hind end and is surrounded by corrugated thickenings of the longitudinal lines; sometimes a small flap is present. The ovejectors are 0·32 to 0·37 mm. long, well developed and contain a few eggs which measure about 0·067 by 0·037 mm.

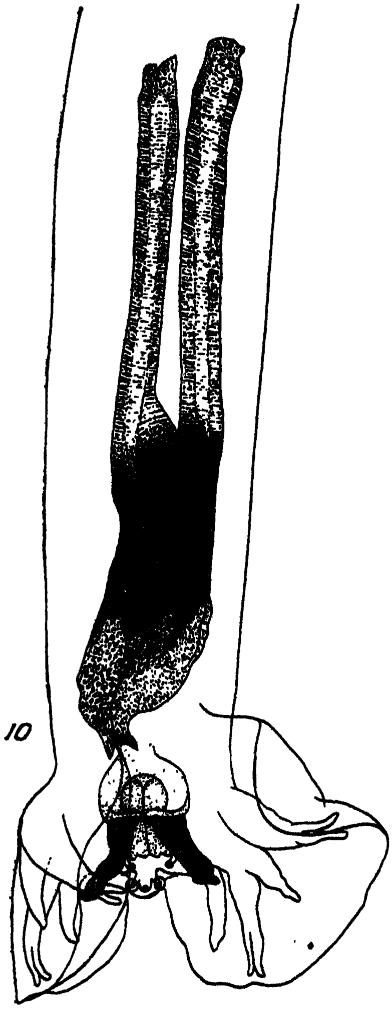
Host—Impala (*Aepyceros melampus*).
Location : Small intestines.
Locality : Kruger National Park.
Types in Onderstepoort Helminthological Collection, No. 2467.

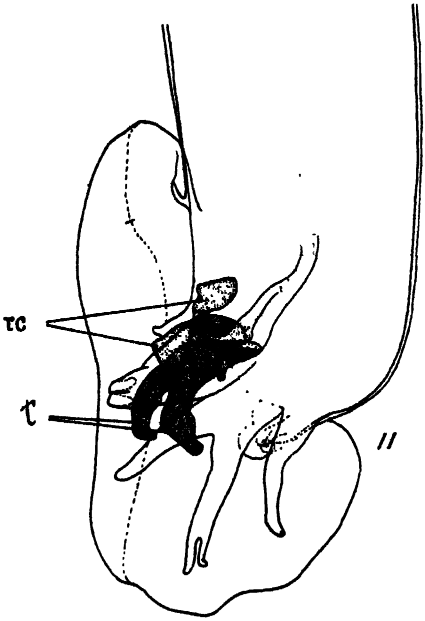
PNEUMOSTRONGYLUS CALCARATUS, MÖNNIG, 1932.

The worms are filiform and the females are thicker than the males. The head is about 0·03 mm. in diameter. Head papillae are very small and difficult to see. The mouth is surrounded by apparently three small lips and leads directly into the oesophagus. The latter is cylindrical in shape and 0·33 mm. long. The nerve ring lies obliquely around the oesophagus, about 0·176 mm. from the anterior end of the body.

The longest portion of a male recovered from the lung measured 29·7 mm. The males are 0·082 to 0·109 mm. wide in front of the bursa. The latter has large lateral lobes, extending forward along the body some distance anterior to the rays (Fig. 10). There is no dorsal lobe. The ventral rays have a common stem about twice as long as the rays themselves. The antero-lateral diverges from the medio-lateral ray, which is fused with the postero-lateral except at their distal extremities. The externo-dorsals arise separately and the dorsal ray is small, flexed in underneath the body towards the ventral side and ends in three papillae (Fig. 11). The margin of the cloacal opening bears a papillae on either side. The spicules are equal and similar (Fig. 10), expanded, and pigmented brown in their posterior halves, only the distal extremities again are unpigmented. They are 0·384 to 0·421 mm. long. There is no gubernaculum but a strongly-developed telamon, consisting of a central unpigmented body which gives rise to a pair of hooklike arms. The latter are each 0·056–0·06 mm. long, conspicuous on account of the fact that they contain brown pigment and project from the cloacal opening. Altogether five male tails were found and the telamon was in this position in all of them. Between the bases of the two arms there is a refractive piece of chitin (Fig. 11) which moves with the telamon as the worm is rolled and is apparently fixed to it.

No characteristic parts of female worms were found. The eggs in the uteri are segmenting and such eggs are also found in the lungs together with others in more advanced stages of development as well as numerous free larvae.





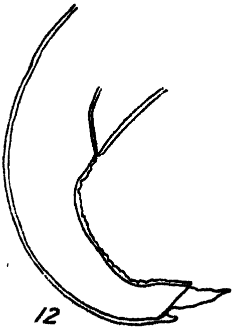


Fig. 10. Pneumostrongylus calcaratus.... Hind end of male, ventral view.
Fig. 11. " " .... Male bursa and telamon, lateral view.
rc—refractive chitin.
t—telamon.
Fig. 12. Pneumostrongylus calcaratus.... Hind end of larva from lungs.

The eggs measure 0·09–0·109 by 0·045–0·052 mm. The larvae are 0·28–0·33 mm. long; the head is simple and has a small mouth. The tail of the larva is characteristic (Fig. 12), it is usually curved ventrad and has a small dorsal spur near the distal extremity which ends in an acute point.

Host—Impala (*Aepyceros melampus*).
Location: Lungs—alveoli and small bronchi.
Locality: Sabie, Transvaal.
Types in Onderstepoort Helminthological Collection No. 2496.

The worm differs so markedly from other known Metastrongylidae, especially in the strong development of the telamon, that a new genus had to be created for it.

Generic diagnosis. Genus *Pneumostrongylus–Metastrongylidae:* body filiform; mouth with apparently three minute lips; buccal cavity absent. Male bursa with large lateral lobes, dorsal lobe absent; ventral rays close together; antero-lateral divergent from the fused medio- and postero-laterals; the latter separate only at the tips; externo-dorsals arise separately; dorsal ray very short, bent in under the body and ending in a few papillae. Spicules equal, stout expanded and pigmented. Gubernaculum absent. Strongly developed telamon present. Oviparous, eggs segmenting when laid.

Transmission Tests.

Faeces cultures of the impala contained only a small number of larvae of *Trichostrongylus*, *Cooperia* and *Strongyloides*. Two lambs which harboured only a few *Trichostrongylus* and *Strongyloides* were infected with these larvae. The first lamb, killed after three weeks, harboured only a few females of *Trichostrongylus*. The second lamb, killed six weeks after infection, had *T. falculatus*, *T. instabilis* and *Cooperia hungi*. The specimens of *T. instabilis* showed the same variations as had been found previously in lambs infected from the waterbuck and described in Part I of this series. A normal type and an extreme variant of these spicules are shown in Fig. 13.

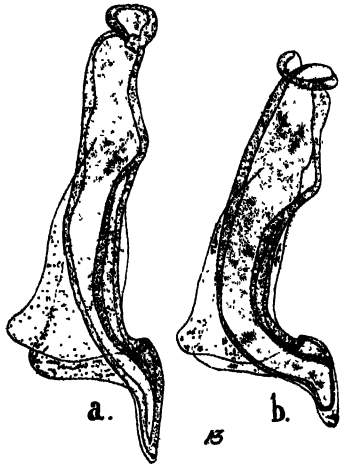


Fig. 13. Trichostrongylus instabilis....... Lateral view of right spicules—(*a*) normal, (*b*) extreme variation.

2. KOODOO (*STREPSICEROS STREPSICEROS*).

This material was from the same source as that from the impala. It contained the following worms:—

From the right ventricle and pulmonary arteries: *Cordophilus sagitta.*

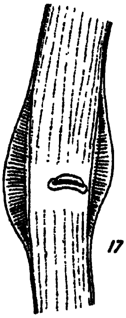
From the abomasum: *Haemonchus vegliai.*

From the small intestine—*Cooperia neitzi*, and from the Colon: *Agriostomum cursoni.*

COOPERIA NEITZI, MÖNNIG, 1932.

Moderately small worms, anteriorly coiled. The cuticle bears 20 to 30 longitudinal striations beginning 0·138 to 0·15 mm. behind the anterior end, which bears transverse striations. The head is 0·043 to 0·048 mm. wide, without any prominent cuticular swelling. The excretory pore is situated 0·44 to 0·53 mm. from the anterior end, near the end of the oesophagus and frequently behind it in the females. A pair of minute cervical papillae are present directly behind the level of the excretory pore. The oesophagus is 0·457 to 0·49 mm. long in the males and 0·49 to 0·57 mm. in the females, surrounded by the nerve ring 0·33 to 0·37 mm. from the anterior end of the body.

The *males* are 8·44 to 9·83 mm. long and 0·14 mm. wide just anterior to the bursa. The latter has large lateral lobes and a small dorsal lobe (Fig. 14). The ventral rays are divergent, the latero-ventral being much larger than the ventro-ventral and both curve forwards. The antero-lateral is straight, wide and pointed, the medio-lateral slender and distally divergent from the antero-lateral, while the postero-lateral is slender and divergent from its middle. The externo-dorsal arises from the base of the dorsal and lies close to the postero-lateral, almost reaching the margin of the bursa. The dorsal gives off two short, lateral branches at its middle and then soon bifurcates into two terminally digitate branches.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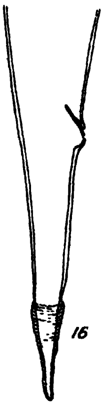
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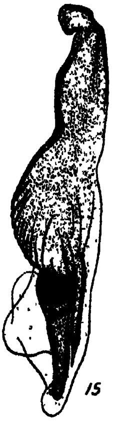
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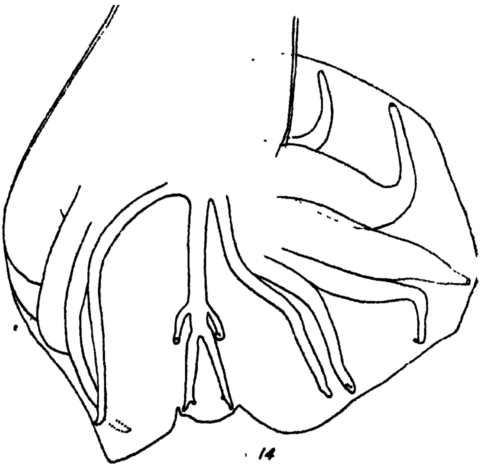
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Fig. 14. Cooperia neitzi.................. Male bursa.
Fig. 15. „ .... Lateral view of left spicule.
Fig. 16. „ .... Tail of Female.
Fig. 17. „ .... Vulvar region.

There are two brown, equal and similar spicules (Fig. 15), measuring 0·195 to 0·217 mm. They have each a pectinate expansion at the middle, followed by a circular depression into which the corrugations extend. The posterior quarter is simple and bears large alae.

The *females* are 10·49 to 12·4 mm. long and 0·108 to 0·13 mm. wide across the vulva, not including the cuticular expansions. The tail is pointed, 0·187 to 0·23 mm. long and the distal quarter is transversely striated, frequently provided with small cuticular alae (Fig. 16). The vulva is situated 2·16 to 2·6 mm. from the posterior extremity and is a transverse slit (Fig. 17) usually accompanied by a pair of lateral alae. The longitudinal striations are interrupted in the vulvar region on the ventral side of the body, but are continuous on the dorsal aspect. The ovejectors are well developed and each is 0·44 to 0·59 mm. long. The eggs measure 0·078 to 0·09 by 0·041 mm.

Host: Koodoo (*Strepiceros strepiceros*).

Location: small intestine.

Locality: Sabie, Northern Transvaal.

Types in Onderstepoort Helminthological Collection No. 2470.

This species is dedicated to my colleague Mr. W. O. Neitz.

Transmission Tests.

Larvae from faeces cultures of the Koodoo were given to two lambs, showing only a slight infection with *Trichostrongylus* and *Strongyloides*. The first lamb, killed after three weeks, harboured a large number of young *Haemonchus vegliai* and several specimens of each of the following: *Cooperia neitzi*, *C. nicolli*, *C. pectinata*, *C. punctata* and *Trichostrongylus instabilis* with variations in the spicules as described above. The origin of the three last named species of *Cooperia* is uncertain. The lambs had not been near cattle or other animals from which they could have become infected with these worms. *C. nicolli* has been found in cattle in South Africa but is very rare. The second lamb, killed after six weeks, harboured only *H. vegliai* and *C. neitzi*.

3. BLUE WILDEBEEST (*GORGON TAURINUS*).

This material, also from the same source as the above, contained only *Haemonchus bedfordi* and the worm was transmitted to one lamb.

4. ELAND (*TAUROTRAGUS ORYX*).

In the beginning of June, 1932, well preserved specimens of worms were received from Mr. J. Walker, Chief Veterinary Officer, Kenya, taken from five eland. It is with much pleasure that I wish to record my appreciation of this kind assistance. The worms were identified as follows: -

Abomasum: *Haemonchus contortus* (2 cases), *H. mitchelli* (1 case).

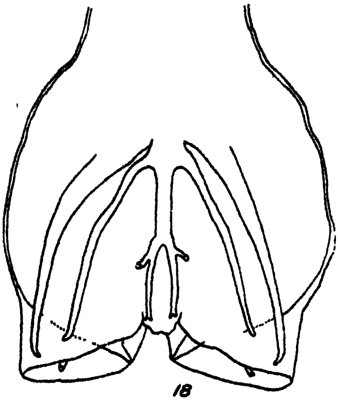
Small intestine: *Impalaia tuberculata* (2 cases), *Cooperia verrucosa* (3 cases), *Cooperia africana* (1 case), *Moniezia expansa* (2 cases), *Avitellina centripunctata* (2 cases).

Large intestine: *Oesophagostomum walkeri* (3 cases).

COOPERIA VERRUCOSA, MÖNNIG, 1932.

Moderately small worms, coiled anteriorly. The head is 0·052 to 0·06 mm. wide and is followed by transversely striated cuticle for a length of 0·157 to 0·187 mm. from the anterior extremity. The rest of the cuticle has 18 to 24 longitudinal striations; of these one in the male and three in the female on either side of each lateral line are punctiform, while the rest have the usual comb-like appearance. Cervical and prebursal papillae are absent. The excretory pore opens just behind the end of the oesophagus, which is 0·457 to 0·53 mm. long. The nerve ring is situated 0·35 to 0·38 mm. from the anterior extremity.

The *males* are 8·2 to 12·6 mm. long and 0·22 to 0·26 mm. wide in front of the bursa. Their bodies are more coiled than those of the females. The bursa is strongly developed and has a small dorsal lobe. The rays show the usual arrangement, the ventrals and laterals being all divergent. The antero-lateral is very stout and the latero-ventral is next in thickness. The externo-dorsals emerge near the base of the dorsal ray, which has a long stem dividing about halfway to the bursal margin; each branch soon gives off a short lateral branch and has a bidigitate distal extremity (Fig. 18). The spicules measure 0·384 to 0·42 mm. in length; they are darkbrown in colour and have a small expansion at the middle which bears corrugations, the latter extending on to the posterior half of the spicule where they are transverse and more numerous than on the expanded portion (Fig. 19). Small dorsal and ventral alae are present.



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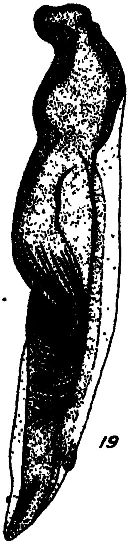

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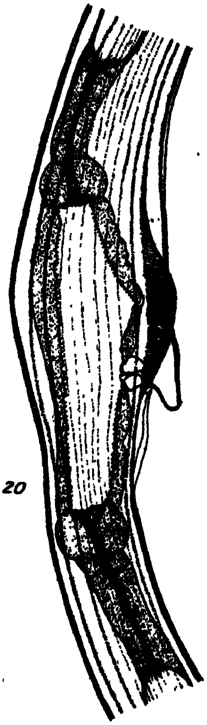

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Fig. 18. Cooperia verrucosa............. Male bursa, dorsal view.
Fig. 19. „ .... Lateral view of left spicule.
Fig. 20. „ .... Vulvar region.

The *females* are 13·85 to 14·8 mm. long and 0·16 to 0·18 mm. wide. The tail is 0·195 to 0·225 mm. long, pointed and bears transverse striations on the last one third of its length. The vulva opens 4·1 to 4·48 mm. from the posterior end. It is covered by an anterior flap (Fig. 20), on either side of which one of the longitudinal striations is raised into an alar expansion. The body is conspicuously thickened in this region. The ovejectors are strongly developed and measure each 0·55 to 0·73 mm. in length. Eggs contained in the ovejectors measure about 0·095 by 0·049 mm.

Host—Eland (*Taurotragus oryx*).

Location: Small intestine.

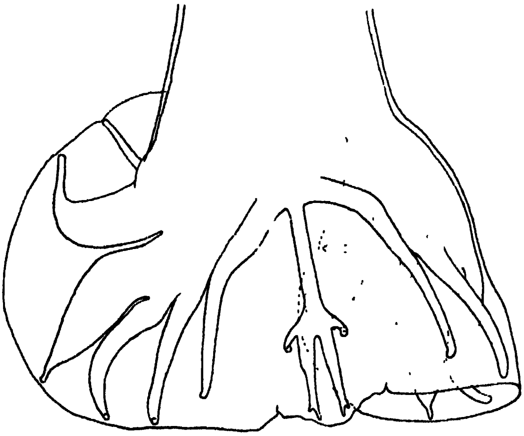
Locality: Kenya.

Types in Onderstepoort Helminthological Collection No. 2478.

COOPERIA AFRICANA, MÖNNIG, 1932.

Only three males of this worm were collected. They are delicate, not much coiled and in a general way resemble specimens of *C. punctata.* They are 4·57 to 4·8 mm. long and 0·11 to 0·12 mm. broad. The head is 0·04 mm. wide, followed by transversely striated cuticle for a distance of 0·07 to 0·078 mm. from the anterior end of the body. The excretory pore opens at or just posterior to the hind end of the oesophagus, which is 0·26 to 0·28 mm. long. The nerve ring is situated at about the middle of the oesophagus. Cervical and prebursal papillae are absent. The cuticle bears 14 longitudinal striations of the usual type.

The bursa has lateral and dorsal lobes, with typical rays (Fig. 21). The ventrals and laterals are divergent, the antero-lateral is thick, but has a slender distal extremity ; the externo-dorsal arises from the base of the dorsal and does not reach the margin of the bursa : the dorsal bifucates at its middle, where it is somewhat thickened and gives off two lateral branches : the terminations of the main branches are bidigitate.



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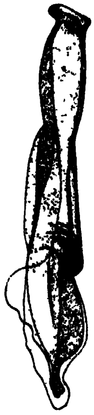

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Fig. 21. Cooperia africana.............. Male bursa.
Fig. 22. „ .... Ventro-lateral view of left spicule.

The spicules are light brown in colour and measure 0·191 to 0·206 mm. They have a small expansion at the junction between the second and last thirds of their length, bearing a few corrugations (Fig. 22). The posterior third bears one large lateral cuticular expansion and there are also smaller dorsal and ventral alae ; the distal extremity is blunt and curved towards the dorsal aspect.

Host—Eland (*Taurotragus oryx*).

Location : Small intestine.

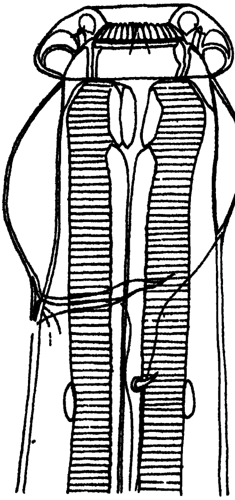
Locality : Kenya.

Types in Onderstepoort Helminthological Collection, No. 2479.

OESOPHAGOSTOMUM WALKERI, MÖNNIG, 1932.

The material consisted of one adult male, two adult females and a fair number of immature males and females of about half the adult size, but already in the final stage of their development.

The anterior part of the body is curved towards the ventral side on account of the large lateral cervical alae. The cuticle bears fine transverse striations, about 0·005 mm. apart. The mouth-collar is well developed (Fig. 23), 0·142 mm. wide in the male and 0·169 mm. in the females, protruding well forward around the external leaf-crown. The cephalic vesicle is prominent ventrally and extends around to the dorsal side also. It extends on the ventral side to the cervical groove, which is situated in the male 0·2 mm. and in the females 0·26 to 0·27 mm. from the anterior extremity and ends on the lateral aspects of the body. The cervical papillae are situated a short distance behind the cervical groove, 0·244 mm. from the anterior end in the male and 0·31 to 0·32 mm. in the female.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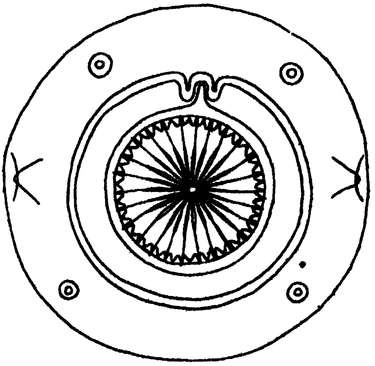
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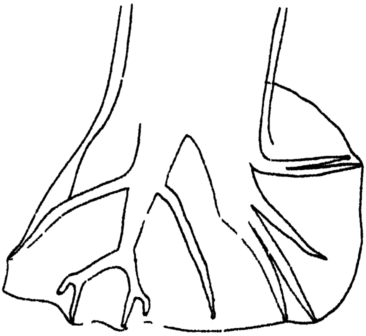
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Fig. 23. Oesophagostomum walkeri....... Anterior end, lateral view.
Fig. 24. „ „ ...... Head, apical view.
Fig. 25. „ „ ...... Male bursa.

The buccal capsule is 0·026 mm. deep, cylindrical or slightly barrel-shaped. In an anterior view of the head (Fig. 24) the buccal capsule shows a very marked indentation of its wall on the dorsal aspect, probably due to the presence of the duct of the dorsal oesophageal gland. Four heads of the immature specimens were cut off to obtain an anterior view and these had respectively 25, 26, 27 and 28 elements in the external leaf-crown and double the number of internal leaf-crown elements. The elements of the external set are long and narrow distally, equal in their length to the radius of the buccal capsule, so that their tips meet in the centre.

The oesophagus is lined with thick chitin forming three short bars anteriorly and then long bars down the whole length of the organ, which is 0·82 mm. long in the male and 0·91 to 0·97 mm. in the females. The nerve ring is situated around the oesophagus 0·3 to 0·34 mm. from the anterior extremity of the body.

The *male* is 12·35 mm. long and 0·256 mm. broad. The bursa is of the usual type. All the rays run out to fine points (Fig. 25). The antero-lateral diverges from the medio-lateral and does not reach the margin of the bursa. The externo-dorsal is slightly curved and reaches near to the bursal margin. The spicules are alate, 0·787 mm. long; in the immature males they measure 0·8 to 0·84 mm.; the gubernaculum is small and triangular in shape.

The *females* are 18·3 and 20·3 mm. long and 0·42 mm. broad at the middle of the body. The tail is 0·27 to 0·31 mm. long, pointed with small papillae near the tip. The vulva is situated 0·897 to 1·04 mm. from the posterior extremity, the smaller measurement being that of the larger of the two worms. The vagina is short and the female organs all resemble those of *O. columbianum*. The eggs in the ovejectors measure 0·1 by 0·056 mm.

Host—Eland (*Taurotragus oryx*).

Location: Large intestine.

Locality: Kenya.

Types in Onderstepoort Helminthological Collection, No. 2477.

5. SPRINGBUCK (*ANTIDORCAS MARSUPIALIS*).

Several springbuck were obtained from the Orange Free State in June, 1932, and in some that died soon after their arrival the following worms were found:—

Abomasum—*Haemonchus contortus* and *Bigalkea albifrontis*.

Duodenum—*Cooperia serrata*, *Trichostrongylus instabilis* and *T. minor*.

Caecum—*Trichuris globulosa*.

Colon—*Agriostomum equidentatum* and *Oesophagostomum africanum*.

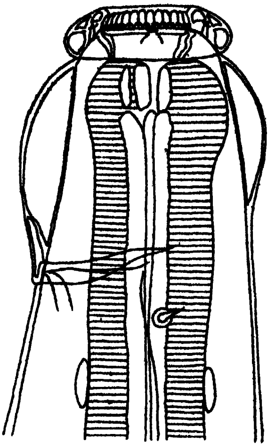
OESOPHAGOSTOMUM AFRICANUM, MÖNNIG, 1932.

This worm is very closely related to *O. walkeri* and it is only by taking into account a number of small but constant differences, that they can be distinguished from each other. It can be easily distinguished from *O. multifoliatum* Daubney, 1932.

There are large cervical alae, causing a curvature of the anterior end of the worm. The cephalic vesicle is exactly similar to that of *O. walkeri*, extending to the dorsal aspect, while the cervical groove ends on the sides of the body (Fig. 26). The cervical papillae are situated 0·217 to 0·244 mm. from the anterior end. The excretory pore opens ventrally into the cervical groove 0·17 to 0·2 mm. from the anterior extremity. The usual lateral and four submedian head papillae are present.

The mouth-collar is well developed but not as high as in *O. walkeri* and is 0·1 to 0·124 mm. wide. The external leaf-crown has 28 to 31 elements, triangular in shape and about half as long as a radius of the buccal capsule. The internal leaf-crown has 56 to 62 elements, the buccal capsule is 0·019 mm. deep, slightly wider anteriorly then posteriorly. Its wall shows an indentation similar to that described for *O. walkeri* but this structure appears to lie deeper down in the wall.

The oesophagus is 0·8 to 0·82 mm. long in the males and 0·88 to 0·91 mm. in the females. The cuticular lining forms, as in *O. walkeri*, three short bars anteriorly, followed by long bars extending to the end of the organ. The nerve ring lies 0·26 to 0·28 mm. from the anterior end of the worm and the distance between the cervical papillae and the nerve ring is greater in this species than in *O. walkeri*.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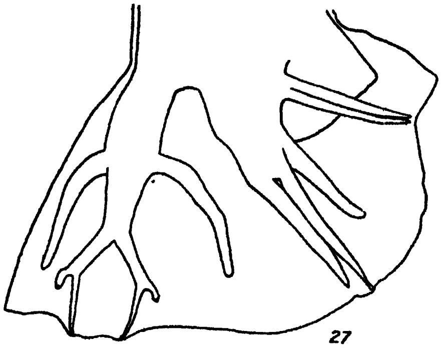
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Fig. 26. Oesophagostomum africanum.... Anterior end, lateral view.
Fig. 27. ,, ,, ... Male bursa.

The *males* are 12·7 to 13·725 mm. long and 0·41 mm. wide at the middles The bursa (Fig. 27) is of the usual type. The common stem of the ventral ray. is very short and there is a notch of variable depth in the bursal margin at the tips of these rays. The antero-lateral is divergent from the medio-lateral, ending bluntly some distance from the margin, the medio-lateral has a slender tip extending right to the edge of the bursa ; the externo-dorsals are blunt and short, while the medial tips of the dorsal branches are long and slender. The spicules measure 0·75 to 0·84 mm. in length and the gubernaculum is small.

The *females* are 13·07 mm. to 19 mm. long and 0·42 to 0·59 mm. wide. The tail is acute, 0·29 to 0·348 mm. long. The vulva opens 0·86 to 0·9 mm. from the posterior end into a short vagina, which together with the rest of the female organs, is of the same type as that of *O. walkeri.* The eggs in the ovejectors measure about 0·15 by 0·075 mm.

Host—Springbuck (*Antidorcas marsupialis*).

Location : Colon.

Locality : Onderstepoort, recently from Theunissen, O.F.S.

Types in Onderstepoort Helminthological Collection, No. 2484.

Transmission Tests.

Several lambs were infected with larvae from these springbuck, repeated infections being given in some cases in the course of several weeks. *Bigalkea albifrontis* and *Cooperia serrata* were transmitted in several cases but neither *Agriostomum equidentatum* nor *Oesophagostomum africanum* was found in the lambs.

TRICHURIS GLOBULOSA (LINST., 1901) RANS., 1911.

As has already been pointed out by Sprehn (1927) *T. globulosa* and *T. ovis* have been badly mixed up in the literature. Perhaps the case is somewhat similar to that of *Ascaridia lineata* and *A. perspicillum*. The writer has examined all the available material at Onderstepoort and found that the *Trichuris* material from the sheep, goat, cattle and wild antelopes is all *T. globulosa*. Perhaps a similar state of affairs may be found to exist in some other parts of the world.

The following list gives the new records of parasites under the names of the hosts, including, besides those mentioned above, a few others that have been collected recently :—

SHEEP (OVIS ARIES).

Cooperia neitzi.
Cooperia nicolli.

IMPALA (AEPYCEROS MELAMPUS).

Trichostrongylus thomasi.
Bigalkea sabie.
Cooperia hungi.
Cooperia hamiltoni.
Impalaia nudicollis.
Oesophagostomum columbianum.
Pneumostrongylus calcaratus.

KOODOO (STREPSICEROS STREPSICEROS).

Cooperia neitzi.
Agriostomum cursoni.

ELAND (TAUROTRAGUS ORYX).

Haemonchus contortus.
Impalaia tuberculata.
Cooperia africana.
Cooperia verrucosa.
Oesophagostomum walkeri.

SPRINGBUCK (ANTIDORCAS MARSUPIALIS).

Bigalkea albifrontis.
Trichuris globulosa.
Trichostrongylus minor.
Oesophagostomum africanum.

DUIKER (SYLVICAPRA GRIMMI TRANSVAALENSIS).

Impalaia nudicollis.
Oesophagostomum columbianum.

(Both through artificial infection.)

SABLE ANTELOPE (OZANNA NIGRA).

Haemonchus contortus.

HARTEBEEST (ALCELAPHUS CAAMA SELBORNEI.)

Haemonchus contortus.	*Agriostomum cursoni.*
Haemonchus bedfordi.	

STEENBUCK (RAPHICEROS RUFESCENS).

Impalaia tuberculata.

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Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 1, Number 1, 1933.

Ozolaimus megatyphlon (Rud., 1819) a little known helminth from *Iguana tuberculata.*

By R. J. ORTLEPP, M.A., Ph.D., Empire Marketing Board Research Officer, Onderstepoort.

THE writer has recently been fortunate in obtaining several well preserved specimens of this interesting parasite, and as it features some peculiar characteristics in its anatomy which have not been figured, the writer has deemed it advisable to attempt a redescription with figures.

These helminths are whitish and relatively stout, the females varying in length from 5 to 6.5 mm. with a maximum thickness in the middle of the body of 0·64 to 0·73 mm., and the males are from 3·5 to 5 mm. long with a thickness of about 0·45 mm. The middle of the body is thickest in both sexes and from here the body becomes attenuated towards both extremities, the attenuation however, becoming more marked towards the anterior end. In the females the tail is short, straight and pointed, whereas in the males it is obtuse and more often curved towards the ventral side. Cuticular annulations are present, and lateral alae are absent in both sexes. The mouth is a vertical slit bounded by two conspicuous and hemispherical lateral lips, which are slightly set off at their bases from the rest of the body (Fig. 1). The anterior edge of each lip is considerably thinner than its remaining portion (Fig. 2), and in consequence when viewed from its side it appears, in sagittal section, as if surmounted by a papilla. The lateral cephalic papillae are very inconspciuous and are situated in the middle of each lip; each is traversed by a thin duct leading from the

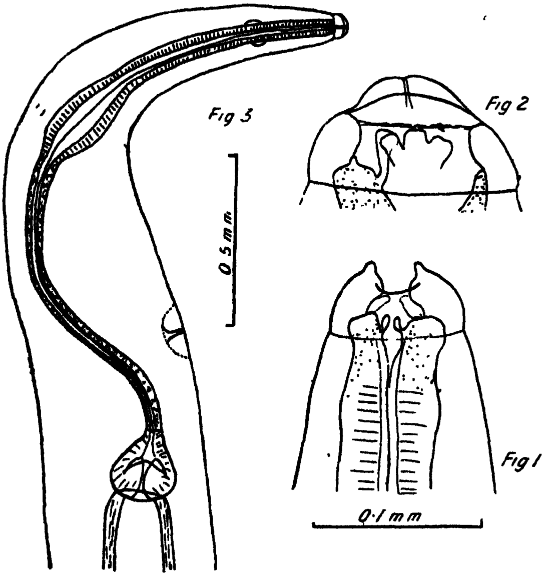

Fig. 1.—Anterior extremity of male, dorsal view.
Fig. 2.—Lateral view of head of female.
Fig. 3.—Oesophageal region of male.

corresponding cephalic gland. Melio-lateral papillae were not evident. The mouth leads into a mouth cavity into which project 3 tripartite cuticular flanges, one from each of the 3 oesophageal segments. It is lined by cuticle but there is no buccal capsule. The oesophagus is relatively very long occupying about 2/5ths of the total body length in both sexes. It consists of two parts, each of which is terminated by a bulb (Fig. 3). The anterior portion is muscular and is thinnest at its proximal end ; it gradually increased in diameter posperiorly where it swells out to form a fusiform bulb about 0·24 mm. long by 0·15 mm. thick ; the posterior oesophageal portion is slightly longer than its anterior portion and is also more slender ; except for its bulb it has a uniform thickness of about 0·05 mm. ; it is semiglandular in nature. The bulb is pyriform in shape and is provided with three cuticular valves characteristic of the Oxyuridae.

The nerve ring is found in the region of the anterior oesophageal portion and is found more or less at the junction of its 1st and 2nd quarters. The excretory pore is prebulbar in position in both sexes, being lodged in the majority of specimens just anterior of the posterior oesophageal bulb ; in some specimens, however, which appear to be much stretched, its position is shifted slightly forwards.

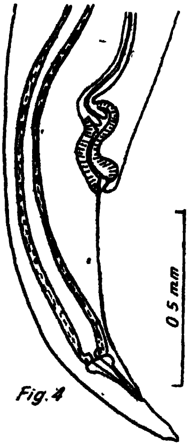

Fig. 4.—Posterior extremity of female.

Female.—The vulva is conspicuous and is found in the posterior quarter of the body usually at about the junction of the 4th and last body fifths ; its distance anterior of the anal aperture varies from 1·13 to 1·27 mm. (Fig. 4). It is a transverse slit whose anterior lip protrudes slightly and overhangs the aperture ; it leads into a short and muscular vagina, about 0·4 mm. long and 0·085 mm. in diameter, which passes obliquely forwards and inwards and has a slight sigmoid shape. The ovejector opens into it through a papilla and is about 1 mm. long and 0·04 mm. thick ; it also passes forwards and its lumen is provided with a cuticular lining. It is followed by the trompe which is of the same length but about three times as thick, and passes backwards parallel to the ovejector. The uterus proper, which follows, is thin walled and at first is dilated to form a small chamber containing a few eggs, after which it splits into the two uteri which bend forwards and together with the two ovaries from a few loops anterior of the vulva. Few eggs are present and these are oval, thin-shelled and contain a partially developed embryo *in utero*; they vary in length from 0·122 to 0·139 mm. with a maximum thickness of 0·06 to 0·064 mm. The tail is short and pointed and varies in length from 0·29 to 0·31 mm., thus forming about 1/20th of the total body length.

Male.—The posterior extremity of the male is cut away ventrally and is generally curved towards its ventral surface. There are only two pairs of papillae, one pair large, ventral and precloacal in position and the other pair small and situated towards the tip of the tail (Figs. 5 and 6). Round the cloaca there are two pairs of appendages, one pair large and dorslateral in position and the other pair small and membranous and occupying a ventrolateral position. Well developed and hyaline caudal alae are present originating just anterior of the insertion of the tail and terminating just anterior of the caudal papillae. The spicule is massive and almost straight and tapers to a fine point; it varies in length from 1·17 to 1·23 mm. with a maximum thickness at its proximal end of 0·025 mm. A conspicuous gubernaculum is present which is V-shaped and projects over the dorsal margin of the cloacal aperture between the two large cloacal appendages. The tail varies in length from 0·13 to 0·146 mm. and forms about 1/38th part of the total body length.

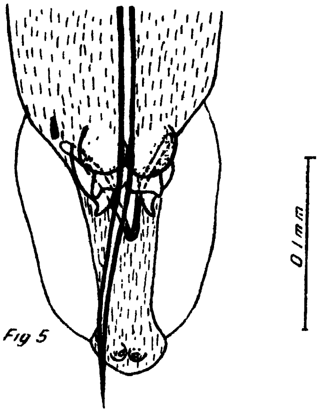

Fig 5

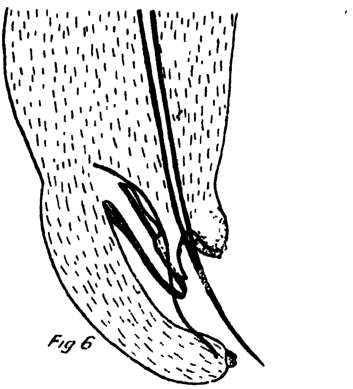
Fig 6

Fig. 5.—Posterior extremity of male, ventral view.
Fig. 6.—Posterior extremity of male, lateral view.

Discussion.—Since Rudolph's original description appeared there is only one record of this helminth, namely, by Dujardin (1845) who examined numerous specimens contained in the Paris Museum and collected from a young Inguana in August, 1841. Unfortunately the scientific name of the host is not given. Dujardin gives a fairly good description but has, unfortunately, missed the details round the male cloaca. There can, however, be no doubt that his specimens are the same as Rudolphi's and the writer's. The peculiar nature of the oesophagus and the presence of only two lips is similar in all, and the spicules in Dujardin's material are of the same length as in the writer's specimens. *Ozolaimus cirratus* (v. Linstow, 1906) was described from material obtained from the same host as the writer's material; the describer differentiated it from Rudolphi's species in that the caudal papillae and appendages were not recorded or figured for the male of this species, and also because the spicule was much shorter—1·25 mm. as against 2·2 mm. in his material. However, to the writer there does not appear to be any doubt as to the identity of these two species. The writer's material has spicules agreeing in length with those in Rudolphi's species, and is also provided with caudal appendages and papillae in the male as recorded for v. Linstow's species; the writer feels confident that v. Linstow's measurements of the spicule are at fault, and that the appendages, etc., on the tail of the male were missed by both Rudolphi and Dujardin. The fact that his material originated from the same host as Rudolphi's and the writer's material lends additional weight to this conclusion. There are, however, slight differences to be recorded in the writer's material; v. Linstow states that in his material the length of the oesophagus was 1/3·9 in the male and ⅓ in the female of the total body; in the writer's material it is relatively longer being about ⅔ths of the body length in both sexes; further he records the presence of five processes ventral of the tail in the males, the most ventral pair of which each carries a papilla; this pair probably corresponds to the 1st pair of caudal papillae described above; the 2nd pair is more dorsal in position and probably corresponds to the large pair of processes described above, and the unpaired process separating these two corresponds to the gubernaculum which forms a process extending dorsally over the cloaca. The small pair of membranous processes described above have probably been overlooked. v. Linstow gives the eggs as 0·098 mm. long by 0·066 mm. broad; this agrees very closely with the measurements given by Dujardin, 0·096 to 0·1 mm. by 0·053 mm., but are much smaller than those measured by the writer.

Affinities.—The presence of a well defined posterior oesophageal bulb with its three valves, the nature of the body musculature, the simple genitalia and shape of the eggs definitely places this genus in the family Oxyuridae Cobbold, 1864; it, however, differs from all the members of this family in two important characteristics, namely, the presence of two large lateral lips and the peculiar structure of the oesophagus; these characters appear to the writer to be of sufficient importance to exclude this genus from the sub-family Oxyurinae Hall, 1916, in which it has always been placed; in consequence, it is deemed necessary to create a new sub-family—Ozolaiminae—for its reception, which sub-family may be diagnosed as follows:—

Oxyuridae. Mouth bounded by two large and simple lateral lips; oesophagus consists of two parts, the anterior muscular and stouter and ending in a fusiform bulb, and the posterior more slender and ending in a pyriform bulb with three valves. Males with a single large spicule, a V-shaped gubernaculum and caudal alae. Vulva in the posterior quarter of the body.

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Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 1, Number 1, 1933.

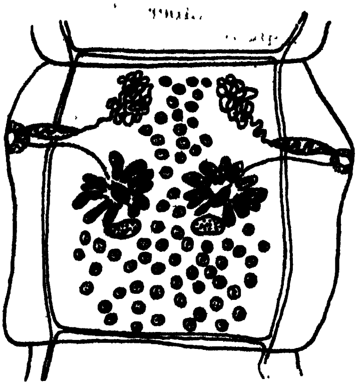
Joyeuxia fuhrmanni Baer, 1924, a hitherto unrecorded Cestode Parasite of the Domesticated Cat in South Africa.

By R. J. ORTLEPP, M.A., Ph.D., Empire Marketing Board Research Officer, Onderstepoort.

A DOMESTIC cat belonging to a member of the staff of this Institute was observed to pass cestode segments in its faeces, and was in consequence placed at the writer's disposal for observation and treatment. An examination of the voided segments showed that they belonged to a member of the genus *Joyeuxia* Lopez-Neyra, 1927, and were very similar to those of *J. pasqualei* (Diamare, 1893), a parasite which had been recorded from domestic cats from various parts of Europe. The cat was treated with two grams of Kamala in milk after fasting, but this failed to remove any worms. It was then decided to keep the cat and utilise the voided segments in an attempt to elucidate the life-history of this parasite. In consequence the segments were collected from day to day and fed to a number of dung beetles belonging to the genera *Hister*, *Aphodius* and *Onthophagus*. The members of the different genera of these beetles, which had been collected from cow dung in the field, were placed in separate tubes containing fresh cow dung mixed with broken up and whole cestode segments: this dung was replaced every day for a week with fresh dung and segments, after which the beetles were fed on untreated dung only. Two beetles of each genus were now killed and dissected every other day from the 10th to the 24th day after their initial feed, i.e., 48 beetles were dissected. A careful search for larval cestodes was made of the various organs and muscles, but no such forms were encountered. This experiment could unfortunately not be continued as the cat had to be destroyed, but so far it appears that if a coprophagus insect is essential in the life-history of this parasite, this insect is probably not a member of the genera mentioned above.

On post-mortem the cat was found to harbour an intense cestode infection, practically the whole length of the small intestine being filled with worms. Over a hundred were collected and these varied in length from about 2 cm. to 6·5 cm. They were allowed to relax and die in cold water and were then fixed in a mixture of equal parts of 70 per cent. alcohol, glycerine and distilled water. Prior to staining and making whole mounts the selected specimens were placed in changes of distilled water to remove all the fixative.

The only record of this parasite is by Baer (1924 and 1927) who described it from *Zibethailurus serval* and *Felis caffra*. This material had been collected by Sir Arnold Theiler and handed over to Prof. O. Furhmann of Neuchatel, Switzerland, for determination. Wittenberg (1932), who has made a comparative study of the members of the Dipylidiinae, came to the conclusion that Baer's species was the same as *J. pasqualei* (Diam.), in that the types of both species agreed with each other in essential characters. While admitting that these two species appear to be closely related, the writer, however, feels that these species must be considered distinct. In all the specimens examined (about 20) the writer failed to find a single specimen in which the vasa deferentia were removed from the anterior margin of the segments, and the testes extending in a zone anterior of the vasa deferentia. The most extreme case seen is that figured, but even here it will be seen that although the testes pass between the coils of the vasa deferentia they do not extent beyond them. According to Wittenberg there is always a space between the vasa deferentia and the anterior margin of the segment in the specimens of *J. pasqualei* examined by him, and that, although this space is larger in the larger than in the smaller specimens several testes are always present in this space anterior of the vasa deferentia. As Wittenberg had ample material at his disposal it appears legitimate to assume that this arrangement of the vasa deferentia and the testes has a specific significance, and as this arrangement is not present in the material at the writer's disposal or in the type material of *J. fuhrmanni*, it would appear that these two species are not co-specific.



The specimens dealt with above agree in essentials with Baer's descriptions. They, however, differ in being larger, the largest specimens being about 65 mm. long whereas Baer's largest specimens measured only 30 mm.; having fewer segments (100 to 130) and in that the scolex does not present an "acorn-like aspect" as described and figured by Baer (1927); this appearance is probably due to contraction as suggested by Wittenberg.

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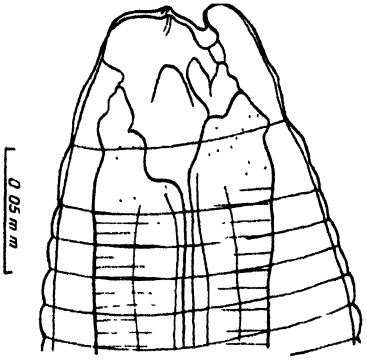
Onderstepoort Journal of Veterinary Science and Animal Industry, Volume I, Number 1, 1933.

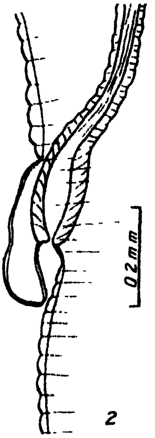
On some South African Reptilian Oxyurids.

By R. J. ORTLEPP, M.A., Ph.D., Empire Marketing Board Research Officer, Onderstepoort.

NUMEROUS oxyurid parasites were collected by Mr. J. H. Power of Kimberley from *Testudo verreauxi*; these were forwarded to this Institute and placed at the writer's disposal for identification. A casual examination showed that several species were present in this material, and on sorting these out it was found that these were two species of the genus *Tachygonetria* Wedl, 1862, one species each of the genera *Thelandros* Wedl, 1862, *Alaeuris* Thapar, 1925, and *Atractis* Duj., 1845, and one species for which it has been deemed necessary to create a new genus. On comparing these species with the descriptions and figures of known tortoise oxyurids it was found that all represented hitherto undescribed species.

TACHYGONETRIA POWERI N. SP. (Figs. 1–6).





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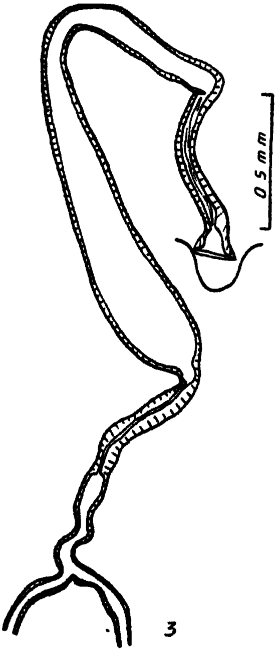

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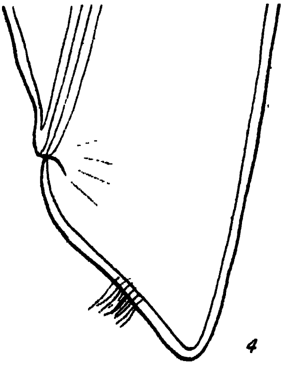
Fig. 1. *Tachygonetria poweri* sp. n. Cephalic extremity.
Fig. 2. *Tachygonetria poweri* sp. n. Vulva and vagina.
Fig. 3. *Tachygonetria poweri* sp. n. Female genitalia.

Some dozen and a half males and females of this species all in good preservation were available for examination. All appear to be fully mature, notwithstanding the fact that none of the females contained any eggs. They are medium sized helminths, bearing long cuticular hairs in some specimens, particularly the females; the males varying in length from 3·7 mm. to 4·6 mm. and the females from 6·1 mm. to 7·3 mm.; they taper in both sexes towards the extremities, the maximum body thickness being found at about the middle of the body; in the males the thickness varies from 0·43 mm. to 0·57 mm. and that of the females from 0·72 mm. to 0·98 mm. The annular striations of the cuticle are about 0·013 mm. apart in the females and 0·012 mm. apart in the males and there are no lateral flanges to the body. The head (Fig. 1) is slightly set off from the rest of the body by a slight constriction, and is provided with three simple lips, one dorsal and two ventro-lateral; these are separated from each other by fairly deep indentations. Only the lateral papillae were seen, and these form slight protuberances in the dorsal half of the lateral lips. Each is traversed by a thin canal. The anterior end of the oesophagus is hollowed out to form an irregularly shaped oesophageal funnel lined with cuticle. Anteriorly the oesophagus carries a thickened rim of cuticle, which carries three tongue-like flanges, one running more or less parallel to the inner surface of each lip; in optical section these flanges look like tooth-like projections projecting into the mouth cavity. The oesophagus is long and thin and is terminated by a rounded bulb with valvular apparatus: in the male it is from 1·8 to 2 mm. long, i.e., about ⅓ of the total body length, and in the female from 2·9 to 3·3 mm. long, i.e., from about ⅖ to slightly less than ½ of the body length. The nerve ring encircles it at about the junction of its 1st and 2nd fifths. The anterior portion of the intestine forms a characteristic swelling in both sexes, and is filled with dark food material.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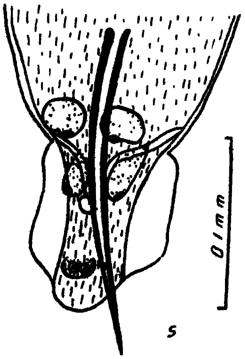

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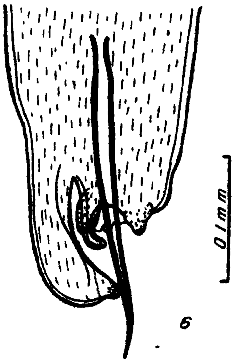

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Fig. 4. *Tachygonetria poweri* sp. n. Tail of female.
Fig. 5. *Tachygonetria poweri* sp. n. Posterior extremity of male, ventral view.
Fig. 6. *Tachygonetria poweri* sp. n. Posterior extremity of male, lateral view.

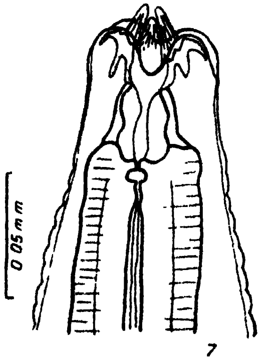
The excretory pore is prebulbular in both sexes.

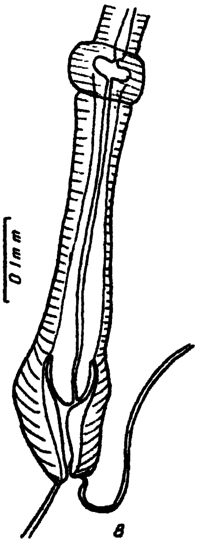
The vulva is situated in the posterior half of the body, roughly at the junction of the 3rd and 4th fifth of the body. Its position is indicated by a flap which arises as a thickening of the cuticle in front of the vulva, and extends backwards over the vulva for about 0·13 to 0·15 mm. (Figs. 2 and 3). The vulva is a transverse slit which leads into a short vagina; this passes into an elongate ovejector, about 0·6 mm. long and 0·055 mm. in thickness extending obliquely inwards and forwards (Fig. 3). The following portion, the "trompe" is J-shaped, the longer limb extending backwards more or less parallel to the ovejector; this portion is enlarged to form a club-shaped egg-chamber some 1·33 mm. long and 0·27 mm. broad at its thickened end; the whole trompe is about 2·5 mm. long. There are two long uteri running forwards somewhat parallel to each other and forming some complicated loops anterior of the vulva and each is about 5 mm. long; these are joined onto the trompe by a short common stem some 0·45 mm. long and 0·05 mm. thick. Small and inconspicuous receptacula semini (0·09 mm. by 0·055 mm.) join the uteri to the short oviducts (0·25 mm.) and these by a sudden thickening pass into the elongate and much convoluted ovaries, some 4 mm. in length. As has already been stated, no eggs were present in any of the females. The tail (Fig. 4) is short and stumpy and varies from 1/40th to 1/46th of the total body length, measuring from 0·15 mm. to 0·17 mm. in length.

The posterior portion of the body of the male is deeply cut out ventrally and is produced backwards dorsally to form a short stumpy tail, 0·108 to 0·117 mm. long (1/33rd to 1/39th of body length) (Figs. 5 and 6). Laterally it carries two conspicuous alae, and towards its tip, on the ventral surface, a single large papilla provided with a double pulp. There are only two pairs of circumcloacal papillae, of which the most posterior pair are elongate. The spicule is large, straight and tapers to a fine point: it is from 0·225 mm. to 0·27 mm. long with a maximum thickness of 0·012 to 0·014 mm. The relatively large gubernaculum has its tip bent ventralwards, and is in the form of a very wide V.

Host: *Testudo verreauxi.*
Habitat: Intestine.
Locality: Niekerk's Hope, Griqualand West.

Affinities.—The presence of three caudal papillae allies it to the members of the genus *Thelandros*, from which members it, however, differs in the absence of lateral and the presence of caudal alae.





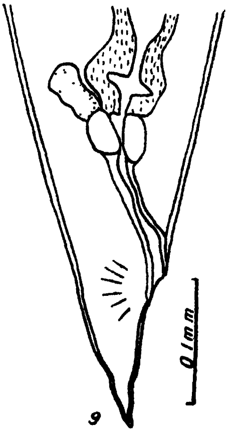


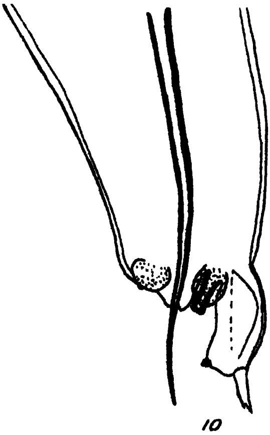
Fig. 7. *Tachygonetria quadrilabiata* sp. n. Cephalic extremity.
Fig. 8. *Tachygonetria quadrilabiata* sp. n. Vulva and vagina.
Fig. 9. *Tachygonetria quadrilabiata* sp. n. Posterior extremity of female.

The outstanding characters of this species are the presence of a vulvular flap, the large spicule, the presence of a double papilla towards the tip of the tail in the male, and the hooked nature of the gubernaculum. As far as the writer is aware none of these characters have been recorded or figured for any of the known species of this genus.

TACHYGONETRIA QUADRILABIATA N. SP. (Figs. 7–11).

The material examined consisted of four males and eight females, all in good preservation. They are rather small worms, the males being from 3 to 3·2 mm. long with a maximum thickness of 0·308 to 0·323 mm. and the females from 4 to 5·3 mm. with a maximum thickness of 0·51 mm. to 0·57 mm.; the body is thickest at about its middle from where it tapers gradually towards both extremities in both sexes.

The head (Fig. 7) is slightly set off from the body, and in the males has a lateral diameter across the base of the lips of about 0·035 mm. and in the females of about 0·05 mm. The cuticle of the head is not traversed by any annular striations, but the rest of the body is and these are about 0·010 mm. apart in the male and 0·015 mm. apart in the female. Lateral alae are absent in both sexes, but both sexes are provided with a few clumps of cuticular hairs, especially evident about the excretory pores. The excretory pore is found just in front of the oesophageal bulb in the females, and at the level of the junction of the bulb and intestine or just posterior to it in the males. There are three conspicuous lips of which the dorsal is large and deeply bilobed; they are all separated from each other by deep indentations. The two lateral papillae are situated one in the dorsal half of each lateral lip and they form a slight bulging from the general contour of the lips. Submedian papillae were not seen. Each lip is strengthened internally by a mass of cuticle carrying three backwardly directed prongs; the innermost of these prongs rests on the anterior margin of the wall of the buccal capsule. Internal to each lateral lip and medially between the two lobes of the dorsal lip there is found a sheet of cuticle which is directed forwards and inwards; these in optical section appear like three large teeth arising one from the inner side of each lip. There is a distinct buccal capsule bounded by thickened cuticle and about 0·025 mm. deep; the capsule has a smaller internal diameter at its anterior end (0·015 mm.) than at its posterior end (0·03 mm.), and the wall of the capsule itself gradually thickens from its anterior towards its posterior margin. The cavity of the capsule is divided into three compartments, confluent centrally, by three large cuticular lamellae arising from the inner surface of the buccal capsule.



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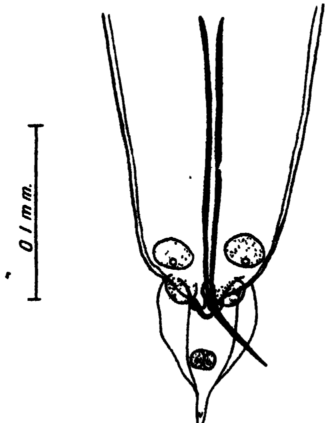

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Fig. 10. *Tachygonetria quadrilabiata* sp. n. Posterior extremity of male, lateral view.
Fig. 11. *Tachygonetria quadrilabiata* sp. n. Posterior extremity of male, ventral view.

The oesophagus is relatively long and thin and is terminated posteriorly by a rounded bulb which is partially sunk into the enlarged anterior end of the intestine. In the males the whole organ is from 1·03 to 1·23 mm. long (1/3·1 to 1/2·3 of the body length) and in the females from 1·75 to 1·94 mm. long (1/2·3 to 1/2·7 of the body length). The nerve ring encircles it about the junction of its 1st and 2nd sevenths.

The vulva is situated in the posterior half of the body and its position is indicated by a small cuticular flap, about 0·035 mm. long, which overhangs it from its anterior face; it is a transverse slit which leads into a short and muscular vagina, about 0·15 mm. long (Fig. 8). The ovejector, about 0·4 mm. long by 0·07 mm. broad, passes obliquely inwards and forwards, and forms a rounded swelling at its junction with the common limb of the uteri. At its junction with the vagina it sends backwards a papillae-like outgrowth extending into the vagina and carrying the genital canal through its middle. The common uterine limb or trompe is long and J-shaped, the larger limb being directed posteriorly parallel to the intestine; it is just over 1 mm. in length with a more or less uniform thickness of 0·035 to 0·04 mm.; no portion of it appears to be differentiated to form an "egg chamber." The two uteri are opposed at first, but the posterior uterus soon bends back on itself and passes forwards parallel to the anterior uterus. Each uterus is about half as long again as the "trompe" and of about the same thickness. A thin oviduct, about 0·3 mm. long, joins the uteri to the large and club-shaped ovaries about 0·8 mm. long. Only three of the females contained any eggs, and in these the number was less than ten in each. The eggs are thin-shelled, oval and smooth and contain a partially developed embryo *in utero*: their average size is 0·15 by 0·07 mm., but they vary in length from 0·14 to 0·152 mm. with a thickness of 0·068 to 0·072 mm. The body is terminated by a short and pointed tail (Fig. 9), 0·132 to 0·154 mm. in length (1/30th to 1/34th of body length).

The posterior extremity of the male is cut away ventrally and is produced dorsally to form a short and alate tail terminated by a spike about 0·02 mm. long (Figs. 10 and 11): the whole tail is from 1/33rd to 1/39th of the total body length, varying in length from 0·08 to 0·09 mm There are three pairs of caudal papillae of which two pairs are large and circumcloacal in position and the third pair is small and situated on the tail just anterior to the origin of its spike: this last pair is small and the papillae are closely approximated to each other. There is only a single spicule which varies in length from 0·215 to 0·225 mm. with a maximum thickness at its proximal end of about 0·015 mm. It is smooth, straight and rounded and tapers gradually to end in a very fine point. The gubernaculum is strongly chitinized, and in the form of a wide V with its pointed extremity straight.

Host: Testudo verreauxi.

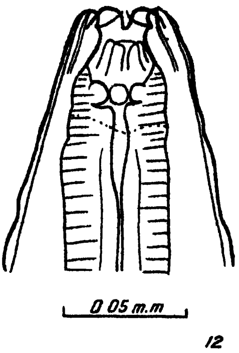
Habitat: Intestine.

Locality: Niekerk's Hope, Griqualand West.

Affinities.—The size and shape of the spicule, the presence of caudal alae, the close approximation of the last pair of caudal papillae, and the presence of a flap over the vulva allies this species to *T. poweri*, from which species it can, however, be easily distinguished by the apparant four lips; presence of a buccal capsule; presence of a spike on the male tail; the unhooked tip of the gubernaculum; the more pointed female tail; and the absence of an egg chamber.

THELANDROS SEXLABIATA N. SP. (Fig. 12–15).

Some half-dozen females of this species were available, and were all in good preservation. They are medium sized worms varying in length from 5·1 to 6 mm. with a maximum thickness, at about the middle of the body, of 0·572 mm. to 0·66 mm.; from this region the body tapers gradually towards both extremities. The head is slightly set off from the rest of the body and is not provided with any cuticular striations; the rest of the body has striae about 0·019 mm. apart, and these extend backwards almost to the middle of the tail. Along each lateral line there is a well developed cuticular ala originating about 0·8 mm. from the anterior end and terminating about 0·35 mm. anterior of the anus; these alae, in the different specimens, have five to seven kinks along their course.



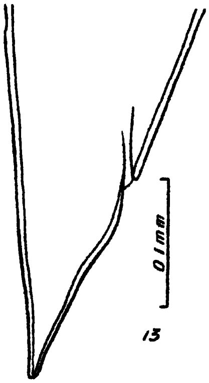
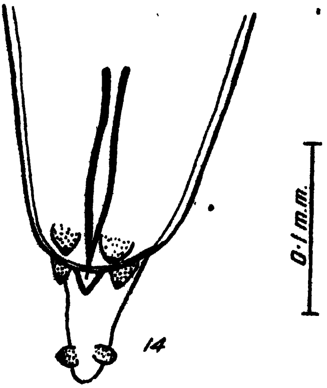


Fig. 12. *Thelandros sexlabiata* sp. n. Cephalic extremity.
Fig. 13. *Thelandros sexlabiata* sp. n. Posterior extremity of female.



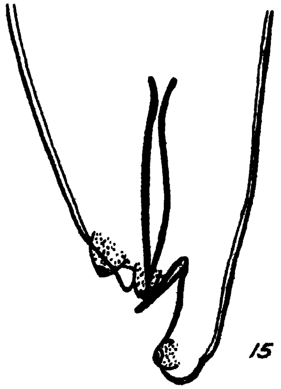


Fig. 14. *Thelandros sexlabiata* sp. n. Posterior extremity of male, ventral view.
Fig. 15. *Thelandros sexlabiata* sp. n. Posterior extremity of male, lateral view.

The excretory pore is prebulbular in position and is generally surrounded by a tuft of cuticular hairs.

The head has a diameter across the base of the lips of about 0·05 mm., and is terminated anteriorly by three markedly bilobed lips (Fig. 12), all the lobes being of the same size and shape. The lateral papillae are represented by a thin duct passing through the centre of the dorsal half of each lateral lip. Sub-lateral papillae were not seen. On the inner surface of the lips, about midway between their anterior and posterior limits there is an annular groove and from this groove a sheet of cuticle extends inwards and forwards into the mouth; in optical section this sheet of cuticle simulates a leaf crown. The anterior end of the oesophagus is slightly expanded and its lumen is enlarged to form a small oesophageal funnel about 0·018 mm. deep; at its base there are found three rounded knobs very prominent in optical section of the head. These are situated one at the junction of each pair of oesophageal segments, i.e., one is ventral and two are lateral in position. Between each pair of knobs and slightly anterior to them there is a small somewhat rectangular flap of cuticle arising from the oesophagus and passing forwards more or less parallel to the inner surface of the lips: these in optical section look like delicate teeth-like structures. The oesophagus is long and delicate and is terminated posteriorly by a rounded bulb; it varies in length, according to the length of the worm, from 2·04 mm. to 2·45 mm. with a more or less uniform thickness, except for the bulb, of 0·05 mm. to 0·055 mm.; the bulb has a transverse diameter of about 0·2 mm.: the whole organ is roughly about 2/5th of the total body length. It is encircled by the nerve ring towards its anterior end, at about the junction of its 1st and 2nd tenths. At the junction of the oesophagus with the intestine the latter forms a saucer-like depression into which the oesophageal bulb is sunk. The 3-oesophageo-intestinal valves project freely into the lumen of the intestine.

The vulva is situated in the posterior half of the body, roughly at the junction of the 2nd and last body thirds; its position is indicated externally by a cushion-like thickening of the cuticle on its anterior face, which cushion may sometimes pass backwards over the vulva in the form of a flap. The vulva is a large transverse slit leading into the short and muscular vagina which passes forwards and inwards, about 0·18 mm. long, and is heavily lined with cuticle. The ovejector is also very muscular, about 0·37 mm. long, and its distal extremity projects into the vagina in the form of a rounded papilla; its proximal extremity is swollen to form a small thickening. The unpaired uterus or trompe is long, about 1·8 mm. long by 0·04 mm. broad and does not appear to have its middle portion enlarged to form an egg-chamber. The paired uteri are each about 3 mm. long and have a uniform diameter of about 0·03 mm. a thin oviduct, about 0·22 mm. long, joins the uteri to the club-shaped ovaries about 1·5 mm. long. The disposition of the different parts of this system inside the body is very similar to that found in *Tachygonetria quadrilabiata.* There are relatively very few eggs present, only three of the seven females examined containing one egg each. These are slightly flattened on one side, oval, smooth, and become morulated *in utero.* They are from 0·12 to 0·125 mm. long by 0·075 mm. broad. Behind the anus the body tapers to form a relatively sharp tail (Fig. 13); this is from 0·154 mm. to 0·171 mm. long—1/37th to 1/33rd of total body length.

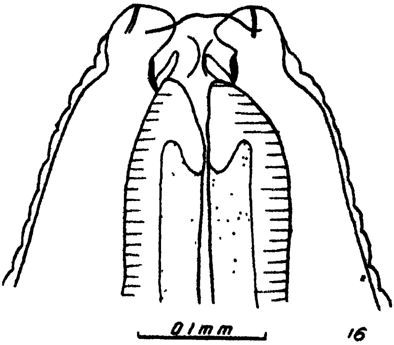
To this species there are assigned some males which do not appear to fit in with any of the other female species found in the collection. These possess most of the general characters described for the females except that the lips are not so markedly bilobed; each lip having only a slight depression in its middle. Otherwise the characters of the mouth and oesophagus are similar, the excretory pore is prebulbular in both and is provided with a bunch of cuticular hairs, and both have well developed lateral alae. These males, which are from 3·6 to 4·1 mm. long, have a maximum body thickness in their middle of 0·374 to 0·418 mm.: the oesophagus is from 1·55 to 1·72 mm. long and occupies almost 2/5th of the body length. The caudal extremity is cut away ventrally; and the tail is roughly trapezoidal and non alate; this latter carries a pair of prominent papillae near its distal extremity (Figs. 14 and 15). It is from 0·063 to 0·072 mm. long and forms from 1/62nd to 1/54th of the total body length. There are two pairs of large circumclocal papillae, and in some specimens there appears to be present a third pair, adanal in position and very small and inconspicuous. There is a single spicule which is straight and thickened towards its middle; its average length is 0·124 mm. but may vary in length from 0·117 mm. to 0·135 mm. The gubernaculum is V-shaped and somewhat inconspicuous.

Host: Testudo verreauxi.

Habitat: Intestine.

Locality: Niekerk's Hope, Griqualand West.

Affinities.—This species appears to show characters which are intermediate between the genera *Tachygonetria* and *Thelandros*, in that it agrees with the former in possessing a gubernaculum and sessile genital papillae, and with the latter in possessing lateral alae; it agrees with both in the absence of caudal alae. As Thapar has rightly stressed, the presence or absence of a gubernaculum in the reptilian oxyurids is not a character to which too great importance should be given as it may be present or absent in members of the same species. Also, the nature of the caudal papillae, i.e., whether they are stalked or sessile, cannot be interpreted too literally as the one type grades into the other. However, because of the markedly bilobed nature of the lips in the female, and the presence of well developed lateral alae in both sexes, the species described above is referred to the genus *Thelandros*. Of the known members of this genus, only two species are known with spicules exceeding 0·1 mm. in length; these are *Thelandros echinatus* (Rud., 1819) in which they are 0·105 mm. long and *Thelandros numidicus* Seurat, 1918 where they reach a length of 0·2 mm. The above described species is easily distinguished from both these species by the presence of lateral alae and further differs from Rudolphi's species by the difference in shape of the caudal extremity of the female, the position of the vulva and consequent direction of the vagina inside the body; and from Seurat's species by the absence of caudal alae in the male.



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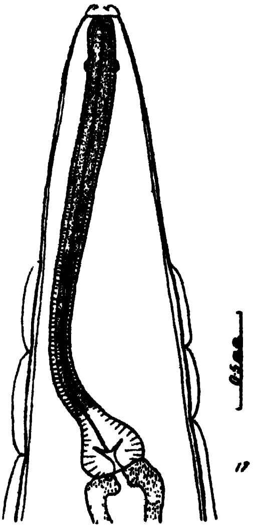

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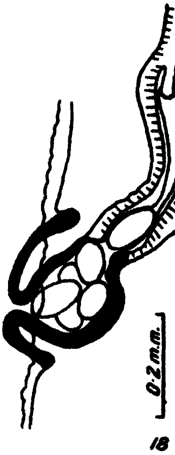
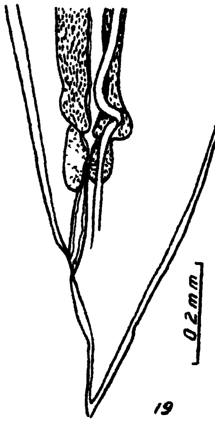
Fig. 16. *Alaeuris conspicua* sp. n. Cephalic extremity.
Fig. 17. *Alaeuris conspicua* sp. n. Anterior extremity.

ALAEURIS CONSPICUA N. SP. (Figs. 16–22).

In the collection there were about four dozen specimens of this species, all in good preservation. They are fairly stout worms, the females being about twice as large as the males ; the latter vary in length from 3·5 to 4·2 mm. with a maximum thickness at about the middle of the body of 0·4 to 0·46 mm. ; from this point the body tapers gradually towards both extremities. The females are from 6 to 8·2 mm. long and have a body thickness at about the middle of from 0·66 to 0·92 mm. The cuticle is finely striated in both sexes, and also carries very conspicuous lateral alae ; these originate from about half-way down the length of the oesophagus and extend to about 0·3 to 0·35 mm. anterior of the ano-genital aperture in the male and to the level of the anus in the female. The alae are unstriated and are festooned to a greater or lesser extent in the individual worms : in some this festooning is fairly regular down to the length of the worm, but this is not usually the case.





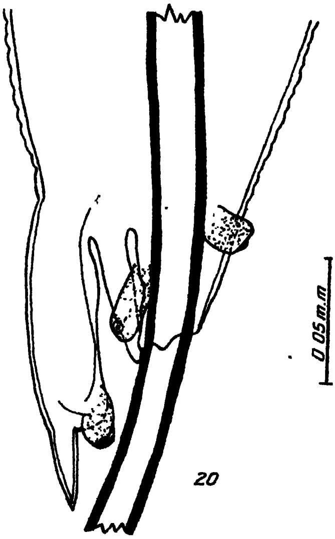
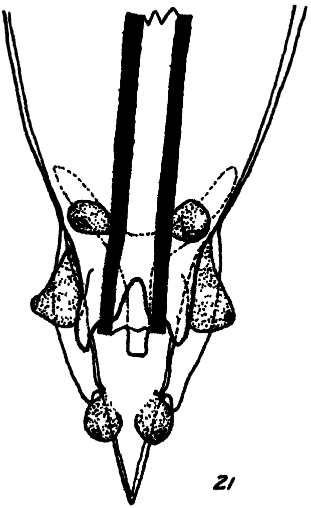


Fig. 18. *Alaeuris conspicua* sp. n. Vulva and vagina.
Fig. 19. *Alaeuris conspicua* sp. n. Posterior extremity of female.
Fig. 20. *Alaeuris conspicua* sp. n. Posterior extremity of male, lateral view.

The head is slightly constricted off from the rest of the body, and is bounded anteriorly by three simple and rounded lips, one dorsal and two ventro-lateral in position (Figs. 16 and 17), there are no submedian head papillae, but the lateral papillae are indicated by a fine duct in each lateral lip. The bases of the lips, on their inner surface, are lined by thickened cuticle, and from the junction of the lips and oesophagus a thickened annular sheet of cuticle projects forwards and inwards into the mouth cavity. The oesophagus is fairly stout and long forming just less than half of the body length in the males and about 2/5th in the females. It varies in length from 1·52 to 1·8 mm. in the males and from 2·27 to 2·7 mm. in the females ; it is somewhat club-shaped, being thickest at its proximal end and thinnest at its junction to the bulb ; except for the bulb it consists of an inner core of a glandular nature traversed by the oesophageal lumen, and of an outer rim of muscular tissue ; the bulb is somewhat pyriform in shape, being just slightly longer than broad ; it is slightly sunk into the broadened anterior end of the intestine, The nerve ring is very inconspicuous and is found towards the anterior end of the oesophagus about 0·23 mm. from the anterior end in the males and from 0·26 to 0·37 mm. from the anterior end in the females. The excretory pore is post bulbular in position in both sexes, being found just posterior to the junction of the oesophageal bulb and intestine in both sexes; in some of the specimens it is surrounded by a bunch of cuticular hairs.

The vulva is very prominent and is found in the posterior half of the body at about the junction of the 3rd and 4th fifths. It is lined by very much thickened cuticle and is sunk into the body to form a transversely elongated chamber (Fig. 18) sometimes found to contain eggs; the lips bounding the transverse aperture protrude slightly above the body surface. The vagina is relatively short, being about 0·5 mm. long and passes obliquely forwards and inwards; it has a thick muscular wall which becomes gradually thicker towards its proximal end, where its lumen is enlarged to receive the papillae-like outgrowth from the ovejector. The uteri consist of a common stem or trompe about 1·75 mm. long, and two long and much coiled uterine branches. The former soon bends backwards and runs more or less parallel to the intestine. The oviducts are short and thin, but the ovaries are relatively massive, long and club-shaped. Eggs were not present in all the specimens, some of the largest even containing none; however, much smaller specimens contained numerous eggs. These are oval, smooth, brownish and thin-shelled, and contain a partially developed embryo *in utero*. They vary in length from 0·113 to 0·12 mm. with a maximum thickness of 0·075 to 0·079 mm. The body is terminated by a short and pointed tail (Fig. 19) from 0·25 to 0·3 mm. in length forming roughly from 1/28th to 1/22nd of the body length.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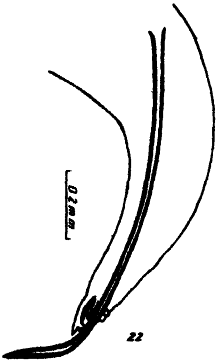

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Fig. 21. *Alaeuris conspicua* sp. n. Posterior extremity of male, ventral view.
Fig. 22. *Alaeuris conspicua* sp. n. Spicule *in situ*.

The posterior extremity of the body of the male (Figs. 20 and 21) is cut away ventrally and is carried back dorsally by a short tail from 1/30th to 1/36th of the body length (0·12 to 0·13 mm. long); it is terminated by a spike about 0·03 mm. long; two plain but conspicuous caudal alae extent from the base of the tail to the origin of the spike. There are three pairs of large caudal papillae, two pairs being circumcloacal in position, the more anterior being ventral, and one pair situated ventro-laterally on the tail at the origin of the tail spike; in addition the lateral margins of the cloacal lips extend backwards as two stumpy processes. A single spicule is present which is relatively very long and varies in length from 1 to 1·27 mm. (Fig. 22); it is broadest at its proximal end, and tapers to end in a sharp point; its distal quarter is slightly enlarged and curved dorsalwards when extruded when it assumes the shape of a sabre. A strongly cuticularized and V-shaped gubernaculum is present.

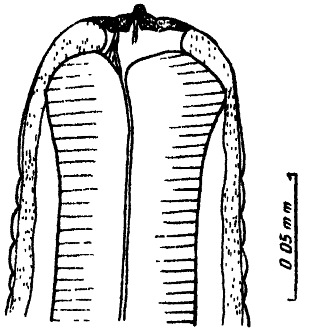
Host: *Testudo verreauxi.*

Habitat: Intestine.

Locality: Niekerk's Hope, Griqualand West.

Affinities.—The presence of caudal and lateral alae, a gubernaculum and three simple lips easily places this species in the genus *Alaeuris* Thapar, 1925. It differs from it, however, in having only three pairs of caudal papillae instead of four pairs. This, however, cannot be considered to be of much weight as the adanal pair is very small or even absent in many reptilian oxyurids. Three species have up to the present been referred to this genus, namely *A. alaeuris* Thapar, 1925, from *Testudo ibera*; *A. iguanae* Thapar, 1925, from *Iguana tuberculata* and *A. hirsutus* Sandground, 1929, from *Iguana rhinolopha*. The above described species is easily distinguished from Thapar's species by its very much larger spicule and by the nature and shape of the vulva. It is distinguished from Sandground's species by the presence of lateral alae and by the complicated nature of its female genitalia.

THAPARIA MACROSPICULUM N.G., N.SP. (Figs. 23–28).



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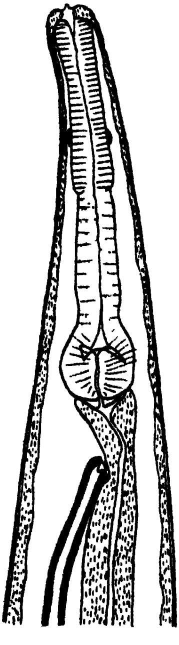

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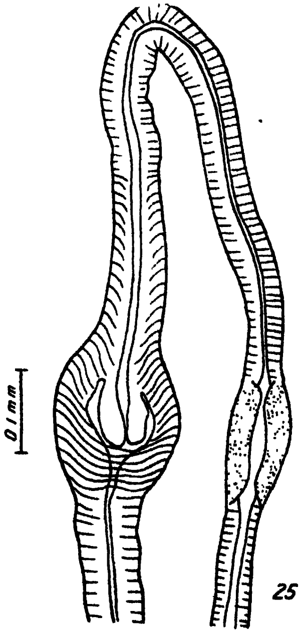

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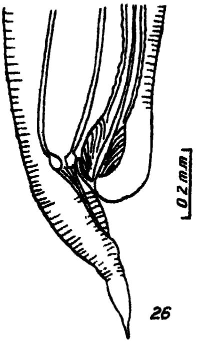
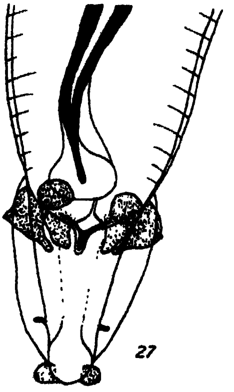
Fig. 23. *Thaparia macrospiculum* g.n.; sp. n. Cephalic extremity.
Fig. 24. *Thaparia macrospiculum* g.n.; sp. n. Anterior extremity.
Fig. 25. *Thaparia macrospiculum* g.n.; sp. n. Portion of female genitalia.

The material available for examination consisted of five males and eleven females, all except one of which were mature. They are medium sized worms the males varying in length from 3·3 to 3·9 mm. with a maximum breadth in the middle of the body of 0·25 to 0·315 mm.; the mature females vary in length from 3·4 to 5·6 mm. with a maximum body thickness of 0·33 to 0·495 mm. The body tapers from the middle towards the extremities in both sexes, the head end of the male being, however, much finer than that of the female. In the female the tail tapers off to a sharp point, whereas in the male it is somewhat trapezoidal in shape. There are three somewhat flattened lips, each of which is slightly bilobed ; on the summit of each dorsal half of the lateral lips there is a conspicuous papilla-like structure on the tip of which opens the duct of the cephalic glands (Fig. 23). No lateral head papillae were observed. On the inner surface of the dorsal lip there is a somewhat rectangular cuticular flange lying close up to the lip and in optical section appears like a spike arising from the base of the lip. There is no buccal capsule. The oesophagus consists of three distinct parts, namely an anterior muscular portion, a middle semi-glandular portion and a muscular bulb (Fig. 24). The middle portion represents the very much elongated neck of the bulb. The whole organ is relatively short being about one-tenth of the total body length in the female and between one-eight and one-ninth in the male. The muscular and glandular portions are separated off from each other by a slight construction, and the former is slightly broader than the latter portion. The whole organ, including the bulb, is about 0·43 mm. long in the males and 0·53 mm. long in the females. The muscular portion measures about 0·18 mm. long in the males and 0·23 mm. in the females with a maximum thickness of 0·045 and 0·063 mm. respectively in the two sexes. It increases slightly in thickness towards its posterior end, and the nerve ring encircles it at about the junction of its second and last thirds. The glandular portion has a maximum thickness of 0·043 mm. in the males and 0·052 mm. in the females ; it has a more or less uniform thickness throughout except that it forms a slight constriction or neck just before it joins on to the bulb. It measures about 0·16 mm. long in the males and 0·19 mm. in the largest females. The bulb is of the usual oxyurid shape and structure and, as a rule, is slightly broader than long ; in the males it is about 0·08 mm. long by 0·085 mm. broad and in the females 0·12 by 0·135 mm. respectively. The excretory pore is situated some considerable distance behind the level of the bulb in both sexes, being found some 0·5 mm. behind it in the males and 0·55 mm. in the females. Lateral alae are absent.





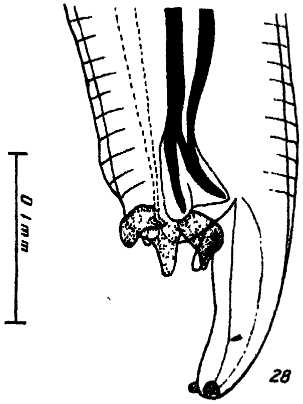


Fig. 26. *Thaparia macrospiculum* g.n. ; sp. n. Caudal extremity of female.
Fig. 27. *Thaparia macrospiculum* g.n. ; sp. n. Caudal extremity of male, ventral view.
Fig. 28. *Thaparia macrospiculum* g.n. ; sp. n. Caudal extremity of male, lateral view.

The vulva is situated near the anus being only about 0·18 mm. anterior to it; its position is indicated by a very pronounced cuticular swelling of its anterior lip (Fig. 26). The vagina is very long, 1·8 to 1·9 mm., and has a uniform thickness of from 0·055 to 0·06 mm.; it passes forwards more or less parallel to the intestine to join a large, oval and muscular sphincter (Fig. 25). This latter is somewhat flask-shaped, consisting of a very muscular bulb, the muscle fibres of which have a semi-circular arrangement, and a neck; these two portions are more or less equal in length, the diameter of the former, however, being about 0·13 mm. and of the latter about 0·07 mm. in the largest female; the centre of the bulb is hollowed out and carries a large papillae, carrying the opening of the genital canal, which extends into it from its anterior face. The ovejector is J-shaped the longer limit being directed posteriorly: it has a more or less uniform thickness of about 0·055 mm. and is terminated by an ovoid swelling; this whole portion is about 0·7 mm. long. The unpaired limb of the uteri is directed posteriorly and has about the same thickness as the preceeding ovejector and is about 0·64 mm. long; except for a short distance the two uteri are opposed, but eventually they recurve and approach one another and finally, together with the two large ovaries, make a few complicated windings about each other in the centre of the body. Relatively few eggs are present in the uteri, most of the females possessing less than ten; however, one female had 26: they are thin-shelled, oval and contain a partially developed embryo *in utero*: they vary in size from 0·09 mm. by 0·064 mm. to 0·102 mm. by 0·062 mm. The tail tapers to a fine point (Fig. 25) and is about 0·35 mm. long; its posterior half is devoid of cuticular annulations.

The posterior extremity of the male is deeply cut out on the ventral side, and the tail itself has a stumpy appearance (Figs. 26, 27 and 28). There are four pairs of caudal papillae, the most posterior pair being situated at the corners of the tail end; the remaining three pairs are grouped around the cloaca, and the first and last of these are large, whereas the middle one is smaller and adanal in position. There are conspicuous caudal alae extending from just in front of the anterior pair of caudal papillae to the base of the last pair of papillae on the end of the tail. An inconspicuous gubernaculum in the form of a wide V is present. The most striking characteristic is the remarkably long and stout single spicule: it extends practically through the whole length of the body, its proximal extremity in some specimens even passing anterior of the oesophageal bulb: it varies in length from 2·57 mm. to 3·4 mm. with a maximum thickness of 0·25 to 0·32 mm.; except for its distal extremity it has a more or less uniform thickness throughout; towards its distal end it narrows down to form a kind of neck after which it broadens out and becomes membranous.

Host: *Testudo verreauxii.*

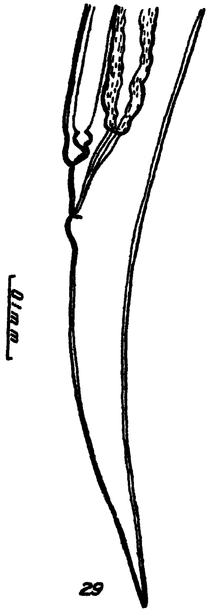
Location: Intestine.

Locality: Niekerk's Hope, Griqualand West.

Affinities.—The shape of the caudal extremity of the male, the disposition and number of the caudal papillae and the presence of caudal alae simulate the corresponding structures found in the members of the genus *Alaeuris* Thapar, 1925, but the nature of the oesophagus, the position of the vulva, the structure of the female genitalia and the extraordinary size of the spicule, these characters together, place this species in a unique position among the reptilian oxyurids; it has in consequence been deemed necessary to create a new genus—*Thaparia*—for its reception, which genus may briefly be characterised as follows: Medium sized worms possessing three lips and a relatively short oesophagus consisting of an anterior muscular portion, a middle glandular portion and a posterior bulb; excretory pore postbulbular; lateral alae absent. Vulva approximated to anus; vagina very long; two uteri and two ovaries. Caudal extremity of male cut and ventrally and continued backwards to form a short truncated and alate tail. Four pairs of caudal papillae, three pairs circumcloacal and one pair towards tip of tail. Single spicule very long and stout extending to or even anterior of the oesophageal bulb. Type *T. macrospiculum* from *Testudo verreauxi*, Griqualand West.

ATRACTIS AFRICANA N. SP. (Figs. 29 and 30).

This species was represented by numerous specimens in different stages of development. They are rather small and slender, the males being from 3·5 to 4 mm. long with a maximum thickness of 0·162 mm. and the females from 4 to 4·5 mm. long with a maximum thickness of 0·22 mm. There is a slight attenuation of the body towards the anterior end in both sexes, which end is terminated in a somewhat bluntly rounded head not definitely set off from the rest of the body. The body of the female is more or less straight and is terminated by a fairly long, straight and pointed tail; in the males the posterior half or third of the body is spirally coiled, and is also terminated by a fairly long and pointed tail.



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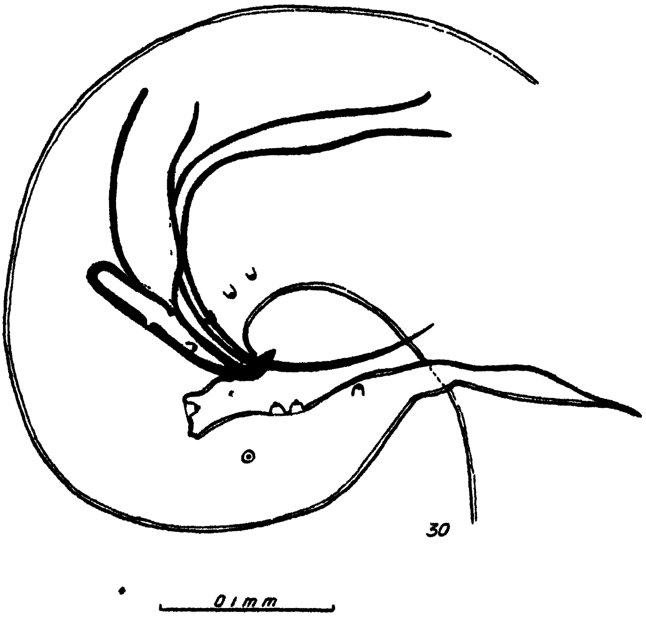

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Fig. 29. *Atractis africana* sp. n. Posterior extremity of female.
Fig. 30. *Atractis africana* sp. n. Posterior extremity of male.

The cuticle is finely striated transversely, and in the oesophageal region is provided with a number of irregularly scattered small bosses; there are no cervical papillae or lateral alae. The mouth is surrounded by six small triangular lips, each of which carries a papillae which protrudes forwards from its tip. There is no buccal capsule. The oesophagus consists of the two parts typical of the genus, namely a muscular anterior portion about 0·36 mm. long in both sexes, and a slightly smaller glandular posterior portion terminating in a bulb; this portion is about 0·34 mm. long in both sexes, and is encircled by the nerve ring near its junction with the anterior portion. The two oesophageal parts are separated from each other by a distinct constriction. The excretory pore is post-oesophageal in position in both sexes; its position is further back in the males than in the females, being found about 0·3 mm. in the males and about 0·2 mm. in the females behind the oesophagus; its aperture is guarded by a number of small and delicate radiating cuticular rods.

The vulva is situated from 0·045 to 0·06 mm. anterior to the anus (Fig. 29) and is lodged behind a small cuticular pad. It leads into a short vagina about 0·03 mm. long which in its turn joins on to the single uterus, whose size depends on the size of the embryos contained in it; it passes forwards ventral of and parallel to the intestine, and is terminated by a small and club-shaped ovary, which is recurved backwards in most cases. The tail is long and pointed and is from 0·41 to 0·46 mm. long.

In the males the posterior extremity is spirally coiled (Fig. 30). There are two very dissimilar spicules. the left being about 0·4 mm. long and terminating in a fine point: at its proximal end it has a thickness of 0·02 to 0·022 mm., and just posterior of this it swells out slightly. The right spicule is stout, hollow and somewhat bottle-shaped with its short neck directed posteriorly; it is from 0·126 to 0·13 mm. long with a maximum thickness of 0·036 to 0·04 mm. at its proximal extremity; its distal extremity is provided with a small aperture leading into the lumen of the spicule. The gubernaculum is straight and strongly cuticularised and has its distal extremity sharply bent ventralwards: it is from 0·11 to 0·125 mm. long; at about the junction of its 1st and 2nd thirds it forms a slight shoulder on its ventral side, and here it attains a maximum thickness of 0·022 mm.; opposite the shoulder, on its dorsal side, there is a characteristic thinning of its cuticular wall. There are nine pairs of caudal papillae. namely two pairs lateral and preanal, two pairs lateral and adanal, and five pairs postanal in position; of these postanal papillae three pairs are approximated towards the ventral line, whereas the remaining two pairs are laterally placed. Behind the last pair of papillae the tail is suddenly attenuated to end in a filiform point; the whole tail is from 0·26 to 0·3 mm. long.

Host: Testudo verreauxi.

Habitat: Intestine.

Locality: Niekerk's Hope, Griqualand West.

Affinities: This species is very closely related to *A. dactyluris* (Rud.) with which species it agrees in the number and distribution of its caudal papillae; it differs from it, however, in its smaller size, much shorter tail in both sexes, slightly larger left spicule, perforated right spicule, the hooked tip of the gubernaculum and the presence of bosses at the anterior end of the body.

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Section IV.

Physiology.

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Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 1, Number 1, 1933.

Studies on the Alimentary Tract of the Merino Sheep in South Africa.

I.—Investigations into the Physiology of Deglutition.

By H. O. MÖNNIG, B.A., Dr. Phil., B.V.Sc., and
J. I. QUIN, D.V.Sc., Veterinary Research Officers, Onderstepoort.

THIS paper is the first of a series of "Studies on the Alimentary Tract of the Merino Sheep in South Africa." The staff of Onderstepoort, realising the tremendous importance of an exact knowledge of all matters relating to the alimentary tract of the sheep, have drawn up a comprehensive programme which includes embryology, anatomy (macro- and microscopic), physiology (physical and chemical), bacteriology and protozoology, to be studied on sheep of all ages under normal conditions, abnormal dietary conditions and pathological conditions due to worm infection and other causes. These investigations will not be conducted in an absolutely systematic manner, but any single question may be studied when a suitable opportunity arrives and the same material is also to be used for the study of other aspects of the problem as far as possible. It is, however, the intention that a special effort should be made to devote as much time as possible to this problem.

INTRODUCTION.

The work recorded here was started on account of difficulties experienced in connection with the chemotherapy of oesophagostomiasis in sheep (see this Journal) with the object of finding a method which would ensure the direct passage to the abomasum of drugs administered per os. The importance of this matter is discussed in the article referred to above. A study on the physiology of deglutition in a ruminant covers a very wide field and the investigations had to be restricted, for obvious reasons, on account of the practical nature of the object in view. The main question was the possibility of administering drugs in the form of powders into the abomasum and the method had to be sufficiently simple in order that it could be applied in practice by farmers handling large numbers of sheep.

A number of investigators have studied the route taken by substances, especially fluids, administered to sheep per os. It is not intended here to enter into a full discussion of their results, especially as this article is concerned with the administration of solids and most of the work done in the past was concerned with nothing more than a determination of the route taken by materials, especially fluids, after ordinary administration. It is, however, clear from the previous investigations, that powders are not regularly swallowed by sheep into the abomasum. Sprehn (1931) records tests made by him with small tablets, weighing 0·2 gm. each, in which he found that such tablets passed directly to the abomasum. These tests were, however, done on a few sheep only and the good results obtained by treating sheep in this way for stomach worms is no proof that the tablets are always swallowed through. Oppermann and Behrens (1932) were not able to confirm the results of Sprehn and the present investigations also do not support them.

The physiology of deglutition, with special reference to the oesophageal groove and the importance of the problem in connection with the administration of drugs has been studied on cattle by Wester (1930) in great detail. This work is of outstanding importance and lays the foundation for all further investigations in this direction, although it will be seen that the sheep used by us frequently reacted quite differently from Wester's cattle. This may perhaps be explained by the fact that the sheep were not unusually tame, while the cattle may have become accustomed to frequent handling and examination through the rumen fistula. Wester remarks that sheep may react like cattle, but that this remains to be proved experimentally.

As far as this paper is concerned, the findings of Wester can be summarised as follows:—

In the very young animal the oesophageal groove closes reflexly during the act of drinking, so that milk and water pass directly to the abomasum.

The reflex for water soon decreases, so that in older calves a portion enters the fore-stomachs and in adult animals the whole quantity, while the reflex for milk persists a long time. However, in relatively young, thirsty animals the reflex comes into action again after a few mouthfuls of water have been swallowed and the abomasum is first filled before the rest passes into the fore-stomachs. In older animals also the reflex closing of the groove can be stimulated by means of suitable fluids more readily when the animals are thirsty than under normal conditions. After drinking a large quantity of water the reflex is sometimes much less readily stimulated. The reflex is initiated in the anterior part of the oesophagus or the pharynx by stimulation of the vagus endings there and the oesophageal groove closes immediately after deglutition. Fluids administered by stomach tube will therefore not cause closing of the groove and will enter the rumen. This reflex can be stimulated by the albumin of milk and possibly also the globulin, and further by sodium salts, especially Sodium chloride and sodium bicarbonate and sugar. The speed and intensity of the reaction depend on the concentration of these substances. Other stimulants tested had little or no effect.

As a practical method of dosing young, healthy cattle, into the abomasum, Wester recommends keeping the animals from water for 24 hours, then dosing with 100–200 c.c. of a 5–10 per cent. sodium bicarbonate solution, followed by the drug together with milk, bloodserum or preferably 5–10 per cent. bicarbonate solution, after an interval which is sufficiently long to allow closure of the groove to take place.

EXPERIMENTAL WORK.

In the following tests a red powder was usually administered after preliminary treatment of the sheep, which were killed after a definite interval, cut open and examined to determine the route taken by the powder. Red mercuric sulphide, carmine and red lead oxide were used, being well visible, relatively insoluble, heavy (except carmine), fine of texture and apparently not irritating to the mucous membranes.

The results are given in tabulated form for the sake of brevity.

The sheep used in these tests were fed on dry hay with a ration of crushed maize and received no green food. Water was always accessible unless it was specially withheld.

TEST I.

No special preparation. Sheep 1–4 dosed with a level teaspoonful of red HgS, 1–2 killed immediately, 3–4 killed 10 minutes after dosing. Sheep 5 given level teaspoonful dry NaCL followed ½ minute later by HgS; sheep 6 similar with interval of 1 minute; sheep 7 given 1 tablespoonful concentrated NaCL solution followed ½ minute later by HgS, sheep 8 similar with interval of 1 minute. Sheep 5–8 killed immediately after dosing.

No.	Age.	Sex.	Cond.	R. cons.	Gas.	Mouth.	Rumen.	Retic.	Om. gr.	Abom.
1..............	4	o	f	+	—	—	2	8	—	—
2..............	4	o	g	+	—	9	1	—	—	—
3..............	6	f	f	—	—	—	2	2	5	1
4..............	4	o	g	—	—	—	1	1	4	4
5..............	6	o	g	+	—	4	—	—	6	—
6..............	4	o	g	±	—	—	—	—	10	—
7..............	4	f	g	+	—	4	—	—	6	—
8..............	6	o	g	—	—	4	3	3	—	—

Remarks.—Age: 1 = lamb; 2, 4, etc. = 2-tooth, 4-tooth, etc.
Sex: m = male, ram; f = female, ewe: o = wether.
Condition: g = good; f = fair; m = moderate, slightly poor; p = poor.
Rumen consistence: + = semi-solid: ± = semi-fluid; — = fluid.
Gas: + = fair to large amount present in rumen and reticulum.

The red powder is considered to consist of ten parts of which the distribution is given under the headings Mouth, if not all was swallowed, Rumen, Reticulum, Omasal groove and Abomasum.

Discussion.—Without stimulation the powder does not regularly pass to the fourth stomach, as has been shown by many previous investigations. Stimulation with NaCl appears to direct the powder through the oesophageal groove. It is not clear whether the consistence of the ruminal contents plays a part; the results with sheep 5–8 seem to uphold Wester's idea that a thirsty animal is more readily stimulated than one that is not thirsty.

TEST II.

In order to see whether thirsty animals are more readily stimulated, these sheep were kept from water for 18 hours. Sheep 9–12 were given a level teaspoonful of NaCl followed by HgS after 1, 2, 3 and 5 minutes respectively and sheep 13–16 were similarly treated but got $NaHCO_3$ instead of NaCl. All were killed 5–10 minutes after dosing.

No.	Age.	Sex.	Cond.	R. cons.	Gas.	Rumen.	Retic.	Om. gr.	Abom.
9..............	8	m	g	+	—	1	—	3	1
10..............	6	f	g	+	—	5	5	—	—
11..............	6	o	f	±	—	8	2	—	—
12..............	4	f	f	+	—	7	2	1	—
13..............	4	f	f	+	—	4	3	3	—
14..............	4	o	f	+	—	2	—	3	3
15..............	4	o	f	+	—	9	—	1	—
16..............	4	o	f	+	—	9	1	—	—

Discussion.—The intervals of two and five minutes between stimulation and dosing may have been too long.

TEST III.

Sheep kept from water 18 hours. All were stimulated with NaCl like sheep 9–12 above. The intervals between stimulation and dosing with HgS were 5 seconds for sheep 17–19, ½ minute for 20–21 and 2 minutes for 22–24. All killed 5 minutes after dosing in this and subsequent tests.

No.	Age.	Sex.	Cond.	R. cons.	Gas.	Mouth.	Rumen.	Retic.	Om. gr.	Abom.
17	6	f	f	+	—	—	—	—	2	8
18	4	o	g	+	—	4	—	—	2	4
19	2	o	m	+	—	—	2	6	2	—
20	4	o	m	+	—	—	8	1	1	—
21	4	o	f	+	—	4	2	2	2	—
22	4	o	p	+	—	4	6	—	—	—
23	4	o	m	±	—	—	6	—	4	—
24	2	o	f	+	—	—	6	2	2	—

Discussion.—The interval of 5 seconds was suitable in two of three cases; the third may have been affected by the condition of the sheep, but it will be seen later that this is not necessarily the case. Sheep 24 was young and should have swallowed well; the interval of 2 minutes may have been too long.

TEST IV.

Sheep kept from water 18 hours. Stimulated with NaCl as before and dosed with HgS after 5 seconds, sheep 25–28 and 1 second, sheep 29–32. The sheep were selected on account of poor condition.

No.	Age.	Sex.	Cond.	R. cons.	Gas.	Mouth.	Rumen.	Retic.	Om. gr.	Abom.
25	8	m	m	+	—	—	1	1	8	—
26	2	f	m	+	—	—	3	2	5	—
27	4	o	f	—	—	5	5	—	—	—
28	4	o	m	+	—	—	1	—	1	8
29	6	f	p	±	—	—	8	—	2	—
30	4	o	p	+	—	—	1	1	—	8
31	4	f	p	±	—	2	2	6	—	—
32	4	o	p	±	—	2	6	1	1	—

Discussion.—There are good indications of sufficient stimulation after 5 seconds. Sheep 27 with fluid contents in rumen poorly stimulated, also 29, 31 and 32, with semi-fluid contents. The others with semi-solid contents well stimulated.

TEST V.

The sheep were kept from water for 18 hours. All dosed with HgS 5 seconds after stimulation with level teaspoonful dry NaCl. Lamb 36 was 2½ months old. Sheep 37 and 38 were each given 2 litres water by stomach tube half an hour before dosing in order to determine the influence of consistence of the ruminal contents.

No.	Age.	Sex.	Cond.	R. cons.	Gas.	Mouth.	Rumen.	Retic.	Om. gr.	Abom.
33............	4	f	m	+	—	—	3	2	5	—
34............	6	f	g	+	—	4	4	2	—	—
35............	8	o	g	+	—	—	—	1	2	7
36............	1	f	f	+	—	—	3	5	2	—
37............	4	o	g	—	—	—	1	1	—	8
38............	8	o	g	+	—	—	1	1	7	1

Discussion.—The result with lamb 36 is quite contrary to expectation. Sheep 37 and 38 swallowed best of all, the rumen and reticulum containing traces only.

TEST VI.

In order to determine further the effects of thirsting and starvation, the sheep were kept from food and water for 22 hours. Dosed as in Test V. Sheep 42 and 43 received each 2 litres water by stomach tube half an hour before dosing.

No.	Age.	Sex.	Cond.	R. cons.	Gas.	Mouth.	Rumen.	Retic.	Om. gr.	Abom.
39............	8	f	g	+	—	—	—	—	6	4
40............	1	f	g	+	—	—	—	—	8	2
41............	4	f	g	+	—	—	—	4	6	—
42............	8	o	g	—	—	4	1	—	5	—
43............	6	f	g	—	—	—	—	—	—	10

Discussion.—Sheep 42 did not swallow properly but the direction was satisfactory. Only sheep 41 with semi-solid ruminal contents did not swallow through completely. In these sheep the starvation did not affect the quantity of the ruminal contents to any appreciable degree and the question arises whether the consistence of the ruminal contents, apart from thirstiness, has an influence. Wester states, that the thirsty animal is more readily stimulated than one that is not thirsty. He stimulates the animal by drenching with 100–200 c.c. bicarbonate solution which, although it is a small quantity for a thirsty bovine, may sufficiently moisten the ruminal contents in the anterior portion to have an effect on the reaction of the oesophageal groove. The above tests appeared at first to indicate that semi-solid ruminal contents are desirable and later, that fluid contents give very good results.

Test VII.

The sheep were kept from water for 24 hours; 44–47 received salt to lick 90–60 minutes and water 60–30 minutes before dosing; 48–51 received 2 litres water by stomach tube 40–60 minutes before dosing. Dosed as above.

No.	Age.	Sex.	Cond.	R. cons.	Gas.	Mouth.	Rumen.	Retic.	Om. gr.	Abom.
44............	2	m	g	±	—	—	1	—	6	3
45............	8	o	g	±	—	—	—	—	2	8
46............	8	o	g	±	—	—	—	—	2	8
47............	8	o	g	±	—	—	—	—	2	8
48............	4	m	g	±	—	—	—	—	2	8
49............	4	o	g	—	+	—	—	—	2	8
50............	6	o	g	—	—	—	—	—	2	8
51............	4	o	g	—	—	5	—	—	1	4

Discussion.—These results were very satisfactory but difficult to explain in view of previous results and Wester's work. The impression was now obtained, that the ruminal contents should be fluid but the sheep still thirsty, if that is possible.

Test VIII.

The sheep were kept from water for 24 hours and allowed to drink one hour to half an hour before dosing. They were apparently not thirsty; only 52 and 53 drank well and 58 and 59 drank a little. Sheep 54 and 55 received each 2 litres water by stomach tube half an hour before dosing and 56–59 were dosed with a level teaspoonful of salt 40 minutes, 20 minutes and 5 seconds before dosing, while 52–55 were dosed as above. In Tests VIII–X carmine was used instead of Hgs.

No.	Age.	Sex.	Cond.	R. cons.	Gas.	Mouth.	Rumen.	Retic.	Om. gr.	Abom.
52............	6	o	g	±	—	—	2	2	2	4
53............	4	o	g	±	—	—	1	1	3	5
54............	6	o	g	±	—	—	10	—	—	—
55............	2	o	g	±	—	—	5	—	4	1
56............	8	o	g	±	—	—	1	—	2	7
57............	4	o	g	+	—	—	2	2	6	—
58............	4	o	g	+	—	—	4	3	2	1
59............	6	f	g	±	—	—	2	2	6	—

Discussion.—The results are so variable that it is difficult to draw any conclusions. Sheep 52 and 53 were thirsty, drank well and swallowed fairly well; 54 and 55 were not thirsty, received water by stomach tube and swallowed to the rumen chiefly. In the case of the other four the results are in favour of semi-fluid ruminal contents and the best effect was obtained in the oldest animal which drank nothing.

TEST IX.

Kept from water 28 hours, then allowed to drink. All drank except 65. Sheep 60 and 61 were dosed 30 minutes; 62 and 63: 45 minutes; 64 and 65: 1 hour; and 66 and 67: 2½ hours after drinking. This test was made especially to see how long the possible effect of drinking would have an influence on the result of stimulation. Sheep 60, 62, 64 and 66 received salt and 5 seconds later Carmine, the others Carmine only.

No.	Age.	Sex.	Cond.	R. cons.	Gas.	Mouth.	Rumen.	Retic.	Om. gr.	Abom.
60	4	f	g	-	—	—	1	—	2	7
61	4	f	g	-	—	—	10	—	—	—
62	8	f	g	±	—	—	1	—	2	7
63	6	f	g	±	—	—	9	1	—	—
64	4	f	g	+	—	—	1	1	—	8
65	6	f	g	–	—	—	8	2	—	—
66	4	f	g	+	—	—	10	—	—	—
67	8	f	g	+	—	—	10	—	—	—

Discussion. Sheep 66 was not properly dosed and this result should be discarded. It seems to be clear that stimulation with NaCl is the most important factor. Sheep 64 with semi-solid ruminal contents, stimulated, swallowed through, while 65 with fluid contents, which did not drink and was dosed without stimulation swallowed to the rumen and reticulum. It is not possible to draw definite conclusions with regard to the effect of drinking.

TEST X.

The sheep were kept from water for 24 hours, then given salt to lick and then water. Only 73 drank a little and all received 2 litres water by stomach tube. Dosed with salt and Carmine 5 seconds later; sheep 68–71: one hour, 72–75: two hours and 76–79: three hours after administration of water.

No.	Age.	Sex.	Cond.	R. cons.	Gas.	Mouth.	Rumen.	Retic.	Om. gr.	Abom.
68	6	o	g	±	—	—	5	3	2	—
69	6	o	g	—	+	—	7	3	—	—
70	8	f	g	—	+	—	6	3	1	—
71	6	o	f	±	+	—	—	—	2	8
72	8	o	f	±	—	—	1	—	2	7
73	6	f	g	±	—	—	1	—	2	7
74	6	o	f	±	—	—	2	—	2	6
75	4	o	f	±	—	—	1	1	4	4
76	4	f	f	—	+	—	6	2	2	—
77	6	o	f	±	+	—	7	3	—	—
78	8	o	f	±	+	—	1	—	2	7
79	8	o	f	±	+	—	8	—	2	—

Discussion.—The gas entered the rumen when the water was administered by stomach tube. Sheep 72–75 dosed two hours after receiving water and, having no gas in the fore-stomachs, swallowed well. Of the others five with gas swallowed badly and two well. Previously sheep 49 also had gas and swallowed well. From this test it seems possible that the gas may have an undesirable effect.

TEST XI.

Another test like No. X was attempted with the intention that the sheep should drink. They were kept from water 24 hours, then given salt to lick for 30 minutes followed by water for 15 minutes. Sheep 80, 83, 84 and 86 did not drink and the others drank a moderate quantity. They were dosed with salt, followed 5 seconds later by red lead oxide, 80–83 one hour, 84–87 two hours and 88–91 three hours after drinking.

No.	Age.	Sex.	Cond.	R. cons.	Gas.	Mouth.	Rumen.	Retic.	Om. gr.	Abom.
80............	8	o	g	+	—	—	7	3	—	—
81............	8	o	g	—	—	—	—	—	2	8
82............	2	o	g	±	—	—	—	8	2	—
83............	4	o	g	+	—	—	—	—	2	8
84............	4	o	g	±	—	—	1	—	9	—
85............	6	o	g	+	—	—	1	1	2	6
86............	6	o	g	+	—	—	9	1	—	—
87............	6	o	g	±	—	—	—	—	2	8
88............	6	o	g	±	—	—	—	—	2	8
89............	2	o	g	±	—	—	—	—	2	8
90............	6	o	g	±	—	—	1	1	8	—
91............	6	o	g	—	—	5	3	2	—	—

Discussion.—Sheep 91 swallowed badly. If drinking has an influence, this may still be evident three hours after drinking. The results were, however, so variable that it appeared desirable to investigate other possible factors.

TEST XII.

The sheep were selected on account of their extremely emaciated condition, which was due to oesophagostomiasis. They had been running on green pastured and were placed in a shed for 24 hours with dry hay and a salt lick, but they took little of either. When they were taken to water it was very cold and raining and none drank. They were dosed with salt, followed after 5 seconds by Carmine in the case of 92–96 and arsenious sulphide in the case of 97–101.

No.	Age.	Sex.	Cond.	R. cons.	Gas.	Mouth.	Rumen.	Retic.	Om. gr.	Abom.
92............	2	o	p	—	—	—	—	—	2	8
93............	6	f	p	—	+	—	6	2	2	—
94............	2	o	p	—	—	—	2	2	2	4
95............	4	f	p	±	—	—	2	—	8	—
96............	8	f	p	—	—	—	—	—	2	8
97............	8	f	p	—	—	8	1	—	—	1
98............	2	o	p	—	—	—	—	—	—	10
99............	4	f	p	—	—	—	—	—	3	7
100............	4	f	p	—	—	—	—	—	2	8
101............	2	f	p	—	—	—	—	—	2	8

Discussion.—The very fluid ruminal contents of all these sheep, except 95, was rather remarkable. In spite of the extremely poor condition they swallowed very well. The impression was again obtained that the fluid consistence of the ruminal contents had an influence, although the effect of drinking was absent.

TEST XIII.

In order to see whether the salt and the red lead oxide could be given mixed, i.o.w. whether the groove closes immediately after stimulation, the sheep were kept from water 28 hours and given salt to lick from 5½ hours before dosing up to 30 minutes before, when they were allowed to drink. Sheep 102 and 103 did not drink. The mixture was administered and the sheep killed five minutes later as usual.

No.	Age.	Sex.	Cond.	R. cons.	Gas.	Mouth.	Rumen.	Retic.	Om. gr.	Abom.
102............	8	o	g	±	—	—	1	1	6	2
103............	8	f	g	±	+	—	10	—	—	—
104............	6	o	g	±	—	—	10	—	—	—
105............	6	o	g	—	—	—	—	—	2	8

Discussion.—No conclusion can be drawn from these results except that the method was not very successful.

TEST XIV.

To determine the route taken by some anthelminthics without previous stimulation as well as tablets and capsules. The sheep were kept from water for 24 hours, received a salt lick during the last 6 hours and were allowed to drink half an hour before dosing. Sheep 111 and 113 did not drink.

Sheep 106 and 107 received ¼ teaspoonful Government Wireworm Remedy

Sheep 108 and 109 received 10 c.c. liquid Government Wireworm Remedy + Carmine.

Sheep 110 and 111 received 0·5 gm. Copper sulphate + 0·5 c.c. nicotine + 30 c.c. water + magenta.

Sheep 112 and 113 received 3 c.c. CCl_4 + 10 c.c. milk + magenta.

Sheep 114 and 115 received 3 c.c. CCl_4 + 10 c.c. 01·lini + magenta.

Sheep 116 and 117 received each two tablets of 1 cm. diameter.

Sheep 118 and 119 received one 100 mg. capsule filled with Carmine.

Sheep 116–119 were stimulated with NaCl five seconds before dosing.

No.	Age.	Sex.	Cond.	R. cons.	Gas.	Mouth.	Rumen.	Retic.	Om. gr.	Abom.
106............	6	o	g	—	—	—	—	—	2	8
107............	8	o	g	±	—	—	1	1	2	6
108............	6	o	g	±	—	—	—	—	—	10
109............	8	f	g	—	—	—	—	—	—	10
110............	2	f	f	—	—	—	—	—	—	10
111............	6	o	f	±	—	—	—	—	—	10
112............	2	f	f	±	—	—	—	—	—	10
113............	4	f	g	±	—	—	—	—	—	10
114............	6	f	g	±	—	—	6	4	—	—
115............	4	f	g	±	—	—	4	2	2	2
116............	6	f	f	±	—	—	—	—	—	2
117............	6	o	g	±	1	—	1	—	—	—
118............	4	o	g	±	—	—	1	—	—	—
119............	6	o	g	—	—	—	—	1	—	—

Discussion.—The Government Wireworm Remedy contains Sodium arsenite and copper sulphate and the sodium salt may have stimulated the groove to close. However, it is known from other tests that this Remedy is not regularly swallowed to the abomasum. Concerning the fluids administered to sheep 108–113; it is apparently usually the case that sheep swallow small quantities of fluid to the abomasum, but the sodium salt (108 and 109) and the milk (112 and 113) may have had an influence. On the other hand, sheep 114 and 115 swallowed the fluid to the rumen. It is rather remarkable that sheep 116 swallowed both tablets to the abomasum, although they have a fairly large size. The capsules in both cases passed into the forestomachs as would be expected. It is obviously out of the question to administer anthelminthics for oesophagostomiasis in the form of capsules or large pills, as they would almost invariably pass to the forestomachs where they will either disintegrate before passing on or they would be passed to the mouth in rumination and probably dropped by the animal.

TEST XV.

The sheep were kept from food and water for 28 hours. The intention was to see what effect starvation had on the abomasal contents and whether thirsty sheep would swallow water to the abomasum, as Wester found to be the case with cattle. Some of the sheep were dosed at the same time.

Sheep 120 and 121 received only salt and 5 seconds later Pb_3O_4. In 120 the ruminal contents were semi-fluid, and in 121 fluid. In both cases the abomasum contained about 50 c.c. fluid ingesta and the powder went to the rumen and a small quantity to the omasal groove.

Sheep 122 and 123 given each 2 litres water with Carmine by stomach tube. This went to the rumen and reticulum as expected. In 122 the abomasum contained a normal quantity of ingesta, in 123 it was almost empty.

Sheep 124 and 125 allowed to drink and killed 20 minutes later after dosing with salt and lead oxide. Both rumen fluid and abomasum about 40 c.c. fluid ingesta. Swallowed to abomasum and omasal groove. Apparently little or none of the water was swallowed to the abomasum.

Sheep 126 and 127 given salt to lick, then water to drink, then dosed with salt and lead oxide and killed. In 126 rumen contents semi-solid, 127 fluid and gas; both had about 40 c.c. fluid ingesta in abomasum. Powder swallowed to rumen in both cases. Here also the water apparently all went to the forestomachs.

Sheep 128 and 129 given each 2 litres water and much air by stomach tube and then dosed with salt and lead oxide. In 128 rumen contained fluid and gas, abomasum almost empty; swallowed powder to reticulum and small quantity to omasal groove. In 129 there was much gas in the forestomachs, abomasum and even the duodenum. The abomasum contained a small quantity of ingesta and the powder. This gives no definite indication whether gas in the rumen hinders closing of the groove. In sheep 129 the excessive amount of gas may have had an abnormal effect.

TEST XVI.

Sheep kept from water 28 hours, dosed a level tablespoonful of salt 8.30 a.m., allowed to drink at 2 p.m. and all drank well. They were then dosed with salt and 5 seconds later with red lead oxide. The intention was to observe any possible factors other than those that were being controlled.

No.	Age.	Sex.	Cond.	R. cons.	Gas.	Mouth.	Rumen.	Retic.	Om. gr.	Abom.
130............	2	f	g	±	--	—	10	—	—	—
131............	2	f	g	—	—	—	—	—	2	8
132............	4	f	g	-	+	-	5	3	2	—
133............	8	o	g	—	+	-	—	1	4	—
134............	8	o	g	-	+	-	1	—	2	7
135............	8	o	g	--	+	—	—	—	2	8
136............	6	o	g			7	—	3	—	—
137............	4	f	g	±	+	—	-	1	2	7
138............	8	o	g	±	-	7	3	—	—	—
139............	4	f	g	±	+	10	—	—	—	—
140............	4	o	g	+	+	—	10	—	—	—
141............	2	f	g	±		—	6	4	—	—

Discussion.—Four of the five sheep with fluid ruminal contents swallowed satisfactorily in spite of gas in three of them. One of six sheep with semi-fluid ruminal contents swallowed satisfactorily and one did not swallow: five had gas. The sheep with semi-solid ruminal contents swallowed to the rumen. These results again indicate that consistence of the ruminal contents is apparently important. Age had no influence, the older sheep swallowed even better than the younger ones. There were no factors that appeared to be important besides those that were taken into account.

TEST XVII.

The object was to determine the effect of various stimulants and sedatives. The sheep were kept from water for 29 hours, dosed a level tablespoonful of salt at 8.30 a.m. and allowed to drink ½–1 hour before treatment.

Sheep 142 and 143 received 0·5 gr. Atropine in 4 c.c. water per os and 1 minute later Pb_3O_4.

Sheep 144 and 145 received 2 gr. Cocaine in 4 c.c. water per os and 1 minute later Pb_3O_4.

Sheep 146 and 147 received 2 gm. Chloral hydrate in 5 c.c. water per os and 1 minute later Pb_3O_4.

Sheep 148 and 149 received 20 c.c. strong coffee per os and 1 minute later Pb_3O_4.

Sheep 150 and 151 received 2 gm. moistened mustard per os and 5 seconds later Pb_3O_4.

No.	Age.	Sex.	Cond.	R. cons.	Gas.	Mouth.	Rumen.	Retic.	Om. gr.	Abom.
142............	2	f	g	—	+	5	5	—	—	—
143............	6	f	g	—	+	—	8	2	—	—
144............	2	f	g	+	+	—	3	—	7	—
145............	2	f	g	±	—	—	1	3	2	4
146............	4	f	g	±	—	—	5	3	2	—
147............	4	f	g	±	+	—	—	—	2	8
148............	2	f	g	—	+	—	10	—	—	—
149............	6	o	g	±	+	5	3	2	—	—
150............	8	o	g	±	+	4	4	2	—	—
151............	2	f	g	±	—	—	10	—	—	—

Discussion.—Atropine acted as had been expected, but the number of sheep used is small. The results with the other drugs are difficult to explain, but the interval may have been too short.

TEST XVIII.

Continuation of previous test. Sheep kept from water for 28 hours and allowed to drink ½–1 hour before treatment.

Sheep 152 and 153 dosed with level teaspoonful sodium acetate and 5 seconds later Pb_3O_4.

Sheep 154–157 dosed with ½ gr. Atropine in 4 c.c. water, 1 minute later NaCl and 5 seconds later Pb_3O_4.

Sheep 158–160 dosed with 0·005 gm. strychine sulphate in 4 c.c. water, 5 minutes later NaCl and 5 seconds later Pb_3O_4.

Sheep 161–162 dosed with level teaspoonful Calcium lactate and 5 seconds later Pb_3O_4.

Sheep 163–164 dosed with 1/6th gr. Physostigimine sulphate and 15 minutes later Pb_3O_4.

Sheep 165-166 dosed with 4 c.c. 6 per cent. Mustard suspension and 5 seconds later Pb_3O_4.

Sheep 167–168 dosed with 10 c.c. 5 per cent. solution of 40 per cent. nicotine and 30 seconds later Pb_3O_4.

Sheep 169–170 dosed with 2 gm. Tartar emetic in 300 c.c. water and 1 hour later Pb_3O_4.

Sheep 171–172 dosed with 2 gm. Tartar emetic in 1,500 c.c. water and 1 hour later Pb_3O_4.

Sheep 173–174 dosed with 3 gm. Barium chloride in 1,500 c.c. water and 1 hour later Pb_3O_4.

No.	Age.	Sex.	Cond.	R. cons.	Gas.	Mouth.	Rumen.	Retic.	Om. gr.	Abom.
152	1	o	m	±	—	—	1	—	2	7
153	1	f	m	—	—	—	—	—	2	8
154	1	f	m	—	—	—	10	—	—	—
155	1	o	m	±	+	—	—	—	2	8
156	1	f	m	±	+	—	6	3	1	—
157	1	o	m	±	+	—	1	—	2	7
158	1	o	m	±	—	—	—	1	2	7
159	1	f	m	—	+	—	—	10	—	—
160	1	f	m	—	—	—	1	1	2	6
161	1	m	m	—	—	—	5	4	1	—
162	1	f	m	—	—	—	5	4	1	—
163	1	f	f	±	—	4	5	—	1	—
164	1	f	f	—	—	—	5	5	—	—
165	1	f	m	—	+	—	6	4	—	—
166	1	f	m	±	—	—	6	4	—	—
167	1	f	f	±	+	—	5	4	1	—
168	1	f	f	±	—	—	5	3	2	—
169	1	f	f	±	+	4	4	2	—	—
170	1	f	f	+	+	—	5	4	1	—
171	4	o	g	+	—	—	5	4	1	—
172	4	o	f	—	—	—	4	3	3	—
173	4	o	f	±	—	—	6	4	—	—
174	4	o	f	±	—	—	3	5	2	—

Discussion.—Sodium acetate acted well as expected. Atropine is not always able to counteract the stimulation of salt, or the route taken by the powder is not influenced by these factors only. The latter view gains support from the other results which indicate that the most important factor has not yet been discovered.

TEST XIX.

In order to test again the influence of starvation sheep 175–180 were kept from food and water and sheep 181–186 from water only for 28 hours. All were allowed to drink ½–1 hour before dosing, but they were apparently not very thirsty, as only 175–177 drank well and a few of the others drank a little. They were dosed in the usual way with salt and 5 seconds later red lead oxide.

No.	Age.	Sex.	Cond.	R. cons.	Gas.	Mouth.	Rumen.	Retic.	Om. gr.	Abom.
175	6	o	g	±	+	—	—	—	2	8
176	8	o	g	—	+	—	—	—	2	8
177	8	o	g	±	+	—	6	4	—	—
178	6	o	g	—	+	—	—	—	2	8
179	8	o	g	+	+	—	1	—	2	7
180	8	o	g	+	—	—	8	—	2	—
181	4	o	g	±	—	—	8	—	2	—
182	2	f	g	+	+	6	—	4	—	—
183	6	o	g	±	—	—	—	—	4	6
184	6	o	g	±	+	—	4	3	3	—
185	8	o	g	±	+	—	—	—	2	8
186	6	o	g	+	+	—	4	3	3	—

Discussion.—These results appear to be in favour of starvation, but when they are taken together with those of Test XV, the findings are not conclusive. Again the presence of gas does not seem to affect the result.

TEST XX.

The foregoing tests, as well as Wester's work, indicate strongly that sodium salts stimulate closing of the oesophageal groove. It was consequently thought that solubility of the particular salt used may be important, since greater solubility should mean more rapid and intensive action on the vagus endings. For this purpose the sheep were kept from water for 29 hours and allowed to drink about half an hour before treatment. All except 199–210 drank. A level teaspoonful of each salt was administered 5 seconds before the red lead oxide in the usual way.

Sheep 187–190 received sodium fluoride, sol. at 30° C about 3/100 gm. H_2O.

Sheep 191–194 received sodium citrate, sol. at 30° C about 11·8/100 gm. H_2O.

Sheep 195–198 received sodium bicarbonate, sol. at 30° C about 11·1/100 gm. H_2O.

Sheep 199–202 received sodium hydrogen phosphate sol. at 30° C about 22/100 gm. H_2O.

Sheep 203–206 received sodium sulphate, sol. at 30° C about 40·8/100 gm. H_2O.

Sheep 207–210 received sodium acetate, sol. at 30° C about 54·5/100 gm. H_2O.

Sheep 211–214 received sodium thiosulphate, sol. at 30° C about 78/100 gm. H_2O.

Sheep 215–218 received sodium nitrate, sol. at 30° C about 95/100 gm. H_2O.

Sheep 219–222 received sodium bromide, sol. at 30° C about 97·3/100 gm. H_2O.

Sheep 223–225 received sodium salicylate, sol. at 30° C about 100/100 gm. H_2O.

Sheep 226–228 received sodium chlorate, sol. at 30° C about 113/100 gm. H_2O.

Sheep 229–231 received sodium iodide, sol. at 30° C about 190/100 gm. H_2O.

Sheep 232–234 received sodium chloride, sol at 30° C about 36/100 gm. H_2O.

No.	Age.	Sex.	Cond.	R. cons.	Gas.	Mouth.	Rumen.	Retic.	Om. gr.	Abom.
187	6	o	g	—	+	—	8	2	—	—
188	6	o	g	±	—	—	8	2	—	—
189	6	f	g	±	—	—	1	1	2	6
190	6	o	g	—	—	—	8	2	—	—
191	8	o	g	+	—	—	8	2	—	—
192	6	o	g	±	—	—	—	—	3	7
193	6	o	g	+	—	—	7	2	1	—
194	6	o	f	±	—	—	8	2	—	—
195	6	o	f	—	—	—	8	2	—	—
196	6	f	g	±	—	—	7	2	1	—
197	6	o	f	±	—	—	8	2	—	—
198	6	o	g	—	—	5	3	2	—	—
199	4	o	g	±	—	—	8	2	—	—
200	8	o	g	±	—	9	1	—	—	—
201	4	o	f	+	—	—	8	2	—	—
202	8	o	g	±	—	—	1	1	3	5
203	6	f	f	—	—	—	8	2	—	—
204	6	o	g	—	—	—	—	10	—	—
205	6	o	m	±	—	5	5	—	—	—
206	6	o	g	±	—	5	5	—	—	—

No.	Age.	Sex.	Cond.	R. cons.	Gas.	Mouth.	Rumen.	Retic.	Om. gr.	Abom.
207	6	o	f	±	—	—	1	—	4	5
208	8	o	g	±	—	—	8	2	—	—
209	6	o	f	±	—	9	1	—	—	—
210	6	o	f	±	—	—	—	—	2	8
211	8	o	g	±	—	—	7	2	1	—
212	6	o	g	—	—	10	—	—	—	—
213	6	o	g	—	—	—	7	2	1	—
214	8	o	g	—	—	3	3	4	—	—
215	4	o	g	—	—	4	3	—	—	3
216	6	o	f		—	—	10	—	—	—
217	4	o	g	—	—	—	7	3	—	—
218	6	o	g	—	—	6	4	—	—	—
219	6	o	g	—	—	10	—	—	—	—
220	8	o	g	—	+	—	2	2	3	3
221	6	o	f	—	—	—	—	10	—	—
222	6	o	f	—	—	—	1	—	3	6
223	6	o	g	±	—	—	7	2	1	—
224	6	o	f	—	—	5	—	—	2	3
225	6	o	g	±	—	—	3	2	4	1
226	6	o	g	—	—	—	10	—	—	—
227	8	o	f	—	—	9	—	1	—	—
228	6	o	f	—	—	—	7	2	1	—
229	6	o	f	+	—	—	8	2	—	—
230	6	o	g	—	—	—	—	—	2	8
231	8	o	g	—	—	10	—	—	—	—
232	6	o	g	+	—	—	—	1	2	7
233	6	o	g	—	—	9	1	—	—	—
234	6	o	f	—	+	—	6	2	2	—

Discussion.—The results show nothing in favour of any particular sodium salt, nor, in fact, do they prove the value of sodium salts as stimulants. At least as good resutls might be expected without any stimulation at all.

TEST XXI.

The object was to test strong alkaline solutions with variation of the interval at the same time. Sheep kept from water for 39 hours, then watered 1-½ hour before dosing.

Sheep 235 and 236 dosed with 4 c.c. N/10 NaOH and lead oxide after 5 seconds.
Sheep 237 and 238 dosed with 4 c.c. N/10 NaOH and lead oxide immediately.
Sheep 239 and 240 dosed with conc. NaCO$_3$ sol. and lead oxide after 5 seconds.
Sheep 241 and 242 dosed with conc. NaCO$_3$ sol. and lead oxide immediately.
Sheep 243 and 244 dosed with NaCl and lead oxide after 5 seconds.
Sheep 245 and 246 dosed with NaCl and lead oxide immediately.

No.	Age.	Sex.	Cond.	R. cons.	Gas.	Mouth.	Rumen.	Retic.	Om. gr.	Abom.
235	4	o	f	+	—	—	10	—	—	—
236	6	o	f	±	—	—	—	—	3	7
237	6	o	f	±	+	—	10	—	—	—
238	8	o	m	±	—	—	1	—	3	6
239	6	o	p	—	+	—	10	—	—	—
240	4	o	f	±	—	—	—	—	3	7
241	4	o	f	±	—	—	10	—	—	—
242	2	o	f	±	—	—	10	—	—	—
243	4	o	f	±	—	—	9	1	—	—
244	4	o	f	±	—	—	6	4	—	—
245	8	o	f	±	—	—	—	—	4	6
246	4	o	f	±	—	—	—	—	9	1

Discussion.—**Again there is no proof that sodium salts act as stimulants or, at least, that stimulation of the vagus is the main factor causing the groove to close.**

TEST XXII.

Similar to the previous test. At the same time the question of pleasant or unpleasant taste was touched, because it was thought that, if sodium chloride was the most active sodium salt, there must be some reason for this and cattle and sheep generally like salt. The sheep were kept from water for 30 hours and watered 1–½ hour before treatment.

Sheep 247 and 248 dosed with 4 c.c. hot conc. NaCl sol. and lead oxide after 5 seconds.

Sheep 249 and 250 dosed with 4 c.c. hot conc. NaCl sol. and lead oxide immediately.

Sheep 251 and 252 dosed with 4 c.c. hot conc. NaCl sol. and mixed with lead oxide.

Sheep 253 and 254 dosed with 4 c.c. cold conc. NaCl sol. mixed with lead oxide.

Sheep 255 and 256 dosed with 4 c.c. cold conc. NaCl sol. and lead oxide + aniseed, immediately.

Sheep 257 and 258 dosed with 4 c.c. cold conc. NaCl sol. and lead oxide $+CS_2$, immediately.

The hot solutions had a temperature of about 60° C.

No.	Age.	Sex.	Cond.	R. cons.	Gas.	Mouth.	Rumen.	Retic.	Om. gr.	Abom.
247............	4	o	m	±	—	—	7	2	1	—
248............	4	o	m	+	—	—	7	3	—	—
249............	2	o	f	±	—	—	—	4	6	—
250............	6	o	m	+	—	—	5	3	2	—
251............	6	o	m	+	—	—	10	—	—	—
252............	2	o	f	+	—	—	1	—	8	1
253............	4	o	f	±	—	—	6	4	—	—
254............	4	o	m	+	—	—	9	1	—	—
255............	4	o	m	±	—	—	6	4	—	—
256............	4	o	m	±	—	—	7	3	—	—
257............	4	o	m	±	—	—	1	—	3	6
258............	4	o	m	±	—	—	5	4	—	1

Discussion.—The hot salt solution had no particular advantage. If the taste plays any part an unpleasant taste would be preferable according to these results. Perhaps a distinction must be made between the taste of the stimulant and that of the drug to be administered.

TEST XXIII.

In view of the results of Sprehn quoted above, the following test was made. Sheep kept from water for 30 hours, then watered 1 hour before treatment.

Sheep 259 and 260 dosed with salt and 1 second later a level teaspoonful of kaffircorn.

Sheep 261 and 262 dosed with salt and 1 second later a level teaspoonful of lead shot.

Sheep 263 and 264 dosed with level teaspoonful of lead shot only.

The kaffircorn has almost round seeds, 3-4 mm. in diameter and the shot was about the same size. The kaffir corn seeds had been stained in eosin to make them conspicuous in the stomach contents.

No.	Age.	Sex.	Cond.	R. cons.	Gas.	Mouth.	Rumen.	Retic.	Om. gr.	Abom.
259	4	o	g	+	—	—	10	—	—	—
260	4	o	g	+	—	—	10	—	—	—
261	4	o	g	+	—	—	10	—	—	—
262	4	o	g	±	—	—	10	—	—	—
263	4	o	f	+	—	—	10	—	—	—
264	4	o	f	+	—	—	—	—	5	5

Discussion.—These results indicate that particles of this size will only exceptionally pass to the abomasum, even after stimulation with salt.

SUMMARY AND CONCLUSIONS.

The possibility of dosing into the abomasum by administering various materials, especially inert powders, after stimulating the oesophageal groove to close, was investigated and the results obtained with 264 sheep are recorded.

The work of Wester was taken as a basis and numerous variations were made in the preparation of the animals, the method of administration and the materials administered. The results are of such a nature that practically no conclusions can be drawn. It is possible that soluble sodium salts stimulates the vagus endings in the pharynx and may thus cause reflex closure of the oesophageal groove, but other important factors, which have not been elucidated, frequently counteract such stimulation or the reflex. It is possible that semi-solid nature of the ruminal contents and the presence of gas in the rumen and reticulum are such adverse factors.

On the whole, the age and the condition of the sheep did not appear to affect the results.

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Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 1, Number 1, 1933.

A Study of the Duration of Motility of Spermatozoa in the Different Divisions of the Reproductive Tract of the Merino Ewe.

By Prof. J. QUINLAN, F.R.C.V.S., Dr.Med.Vet., D.V.Sc., Sub-Director of Veterinary Services and Animal Industry;
G. S. MARÉ, B.Sc (Agric.) (S.A.); and
L. L. ROUX, M.Sc. (Ill.), Research Officers in Sheep and Wool.

INTRODUCTION.

In a previous paper the authors (1932) discussed the vitality of spermatozoa in the genital tract of the Merino ewe, with special reference to its practical application in breeding. During that series of experiments it became apparent that the spermatozoa survived longer in the cervix than in the other divisions of the reproductive tract in the Merino ewe. It appeared that the secretions of the vagina and the divisions of the genital tract cranial to the cervix were, in comparison with the cervix, definitely unfavourable to the life of spermatozoa. In the cervix alone did the secretion appear to be favourable. It was therefore suggested that the cervix of the Merino ewe acts as a reservoir for spermatozoa, where they are maintained under favourable conditions pending ovulation and the arrival of an available ovum in the Fallopian tube.

The present experiments were undertaken to ascertain whether, by isolating spermatozoa in the uterine horns and the Fallopian tubes, an accurate estimate of the duration of their vitality in the different divisions of the reproductive tract could be made; that is to observe, from a point of view of motility, whether the secretions from the vagina, uterus, uterine horns, and Fallopian tubes act detrimentally as compared with the secretion of the cervix.

LITERATURE.

Although a considerable amount of work has been done on the vitality of the spermatozoon in laboratory animals, especially the rabbit, the guinea pig, and the rat, the literature is not rich in references to work done on sheep. No references can be found to the particular aspect now under consideration, namely the motility of the sperms at different levels of the reproductive tract.

The literature relevant to the vitality of the spermatozoon in the genital tract has been discussed by the authors in the paper referred to above, so that it is considered unnecessary to repeat the discussion in detail here.

Hammond and Asdell (1926) have shown that there is some unfavourable influence on the vitality of rabbit spermatozoa by the female genital secretion. These authors point out that spermatozoa taken from the male epididymis retain their vitality for three days, while those taken from the vagina of the female after copulation retained their fertilizing power for only 30 hours. Walton Hammond and Asdell (1928) found that spermatozoa collected from the epididymis of the killed rabbit retained their fertilizing power longer when kept in vitro than those collected from the vagina immediately after copulation. Yochem (1929) has studied the life of the spermatozoon in the genital tract of the female guinea pig and rat during oestrus and also during the interoestrous period. He found that in the guinea pig sperms artificially inseminated during the oestrous period survived somewhat longer than those inseminated during the interoestrous period. In the case of insemination during oestrus, motility was maintained for 41·5 hours in guinea pigs and 12·5 hours in rats. The duration of life of the sperms artificially injected during the interoestrous period was 36 hours in the guinea pig. The sperms of rat semen injected into guinea pigs survived only 4·5 hours, and guinea pig sperms into rats only 11 hours.

Löw (1902) has observed in the case of rats that the vaginal secretion of the female is unfavourable to the life and the motility of spermatozoa, while the uterine secretion is favourable.

Sabotta's (1895) observations on the mouse have shown that the greater majority of the sperms in the uterus are non-motile 6 to 10 hours after coitus. Kugota (1929) made observations on the influence of uterine secretions on the life and motility of the spermatozoa of the mouse during different periods of the oestrous cycle. He concluded that the secretions had different influences at different periods. He says :—

> " In der zweiten Periode wirkt er höchst günstig. Diese Wirkung beginnt schon in der ersten Periode und ist in der dritten Periode plötzlich sehr gering. Diese Einwirkung auf die Lebensdauer der Spermatozoen scheint um so günstiger zu sein, je stärker der Uterussaft konzentriert ist. In der vierten Periode und im Dioestrum können wir weder eine günstige noch eine nachteillige Wirkung finden."

The division into five periods was made by Kugota according to the microscopic appearance of vaginal smears and sections of the vaginal wall.

Hammond (1930) working with rabbits has shown that sperms taken from the vagina and maintained outside the body at different temperatures may retain fertility at 35° C. for 14 hours ; at 10° C. for 96 hours, and at 0° C. for 16 hours. Walton (1930) also working with rabbits, in co-operation with Hammond, has taken sperms from the epididymis of the male and maintained them outside the body at different temperatures. His results regarding fertility were more or less similar to those of Hammond. Above body-temperature the spermatozoa were rapidly destroyed ; at 37° C. to 40° C. the maximal survival was about 13 hours. There was an increasing prolongation of survival as the temperature was lowered until a maximum of about 7 days at 15° C. was reached.

These experiments were done with the object of testing the effects of temperature on spermatozoa, and the work of both authors is confirmatory, but on analysing their results from a point of view of the present work, it is evident that the spermatozoa taken from the vagina by Hammond were less vital than those taken from the epididymis by Walton.

Hutschenreiter (1915) has shown that motility of spermatozoa of the stallion has usually ceased after 4 hours in the vagina of healthy mares during oestrus, while in the uterus sperms survived up to 10 hours. He found that spermatozoa survived somewhat longer in the vagina during the interoestrous period than during oestrus.

Quinlan, Maré, and Roux (1932) have shown that spermatozoa live longer in the cervix than in the other divisions of the genital tract in the merino ewe. These authors have further observed that sperms obtained from the ram without having come into contact with vaginal secretion survive longer in vitro than sperms taken from the vagina after normal copulation. The maximum time of survival of spermatozoa, taken from the vagina of sheep immediately after copulation, and kept in sterile pipettes at room temperature appears to be about 48 hours; while in semen taken from the same ram without admixture with vaginal secretion and kept under similar environmental conditions, the spermatozoa have survived 56 hours.

In the case of the human sperm it appears to be recognised that the duration of life varies in the different divisions of the female genitalia. Giles (1919) states that spermatozoa in the vagina die within one hour after coitus; in the cervical canal they may be found 2 to 5 days after coitus; in the fundus they are frequently found 24 hours after coitus and occasionally after several days. More cranially, that is in the Fallopian tubes, their normal behaviour is unknown. Haussman (1879) and Hühner (1913), quoted by Giles, maintain that the life of the sperm in the vagina is not longer than a few hours. Hühner (1913) has shown that living sperms have been found in the cervical canal after 15 to 24 hours only in 11·6 per cent. of cases; after 2 to 5 days in 20 per cent. of cases, and after 1 to 12 hours in 45·9 per cent. of cases. The same author's examination for sperms in the uterus have shown living spermatozoa in 27 per cent. of cases after 1 to 12 hours; in 16·7 per cent. after 15 to 24 hours, and in 6·3 per cent. after 2 to 7 days.

METHOD.

The work was carried out during the months of November, 1931, and February, 1932, at the School of Agriculture, Middelburg, Cape Province. The rams used, namely W. 31, T. 413, and T. 417, had been extensively employed by the authors in previous experiments (1931. 1932). Their fertility records were known to be highly satisfactory, as is shown in Table II. They were in good, hard, breeding condition during the time these observations were being carried out. The ewes were full-mouth sheep selected from the flock at the School. They were in good, breeding condition and appeared to be clinically normal. Their previous breeding record was known.

The ewes were tested twice daily for oestrus, at 6 a.m. and 5 p.m., with vasectomised teasers.

Only sheep which allowed copulation without restraint were used for observation. The sheep to be used were brought to the Laboratory immediately before service was allowed. Three services were allowed each ewe. The services followed in rapid succession and were completed in less than 15 minutes. Immediately afterwards the ewe was caught and the hind extremity elevated. Two samples of semen were withdrawn in sterile glass pipettes, which were introduced along the ventral wall of the vagina to its cranial extremity. The amount of semen collected varied from about 1 to 1·5 cm. in each pipette.

After collection of the semen the sheep were taken to the theatre for operation. The wool had been shorn from the left flank prior to service, so that as little time as possible was lost between copulation and actual insemination into the uterus and Fallopian tubes. When observations were first begun several sheep were operated upon after copulation before withdrawal of the semen. The vulvar lips were clamped to retain the semen during operation. The ejaculate was withdrawn only when the uterus was exposed. All these results have been disregarded in this series of observations, as it was considered that the semen had been too long in contact with vaginal secretion before final injection into the selected site in the genital tract, namely, the apex of the uterine horn and the Fallopian tube.

The sheep were anaesthetised by an intrajugular injection of chloral hydrate (10 per cent. with ·9 per cent. saline solution). The amount of chloral hydrate injected is graduated according to the weight of the sheep. This method of anaesthesia is highly successful. It has been used very extensively in this country for major surgery in sheep. [De Kock and Quinlan (1927); Quinlan, Maré and Roux (1930).]

The genital apparatus was exposed through a laparotomy in the left flank. The left uterine horn and left Fallopian tube were withdrawn. At first the intention was to isolate the left horn and the left tube by ligation and subsequent section. After a couple of trials this method of operation had to be abandoned, as impracticable. In the case of the tube the operation produced no pathological change in the mucosa, but the horn, as a closed sac (having been ligated and sectioned cranially and caudally), became filled with fluid so that the environment of the sperms between injection and examination could not be regarded as normal. This procedure had to be modified so that the normal conditions of the right side of the genital tract, which was used as a control, were simulated as closely as possible. The left tube was caught close to the uterine extremity in a small artery forceps and crushed. It was ligated with fine silk on either side of the forceps. The forceps was then removed and the tube sectioned in the crushed area. During the application of the ligatures care was taken that blood vessels in the mesosalpinx were not included.

The semen was now transferred to the tube by introducing the pipette deeply into it through the abdominal ostium and blowing out the semen. The introduction of semen into the horn was done by puncturing the left horn close to its apex with the point of the pipette. The semen was then blown into the lumen of the horn.

The semen was in every case controlled microscopically for activity of the spermatozoa at the time of injection. Further it was retained and examined from time to time for survival of the sperms in vitro. After injection of the semen the uterus was replaced in position. The laparotomy wound was closed by suturing the peritoneum and muscles with No. 1 cat-gut, and the skin with No. 2 suture silk. The sheep were then placed in a shed to await the time of observation. All the sheep had fully recovered from the effects of anaesthesia after 3 hours.

The sheep were killed by bleeding at intervals of 6, 9, 12, 15, 18, and 24 hours after operation. The abdomen was opened through a prepubic mid-ventral incision and the different compartments of the genitalia immediately clamped off with suitable forceps so as to prevent wandering of spermatozoa on the right or control side of the reproductive tract after death of the animal. The genitalia were not removed from their attachments. The different divisions were then opened, fresh preparations were made on glass slides and immediately covered with a cover slip. The microscopical observations for living sperms were all carried out in the natural secretion.

The preparations were immediately submitted to microscopical examination for living spermatozoa. Smears were also made and later examined for morphological changes.

It is realised that this method of examination presents disadvantages since it does not simulate the normal environmental conditions within the genitalia. However, there was little chance of sperms which were alive at the time of slaughter failing to survive the short interval between the death of the sheep and microscopical examination. It is taken, therefore, that dead sperms seen on microscopical examination of fresh preparations were actually dead before the secretions containing them were removed from the genitalia. The examinations were done at the Grootfontein School of Agriculture, at room temperature which varied between 72° F. and 84° F. where the observations were carried out.

The results of the experimental observations are summarised in Table I. They are, however, of sufficient interest to discuss them in some detail first.

The sheep used for observation of the spermatozoa six hours after operation was about 24 hours in oestrus when served. The ovary had not ovulated at the time of slaughter.

Living sperms were found in all divisions of the genitalia both on the operated and control sides. There was, however, a greater percentage of living sperms in the control side: in the horns 68 per cent. as compared with 44 per cent., and in the tubes 40 per cent. as compared with 21 per cent. In the vagina only about 10 per cent. of the sperms were motile. In the cervix spermatozoa were very plentiful: about 55 per cent. being motile. The pars indivisa of the uterus contained relatively few sperms in comparison with the cervix: 71 per cent. were motile.

The sheep used for observation of the spermatozoa nine hours after insemination was about nine hours in oestrus when served. The ovary had not yet ovulated at the time of slaughter. Sperms were numerous in the vagina; about 50 per cent. were motile. Sperms in the cervix were also very numerous, 90 per cent. being motile. A few motile sperms were seen in the pars indivisa. A few non-motile sperms were seen in the control horn; no motile sperms were seen. Spermatozoa were very rare in the operated horn: only two intact sperms were seen of which one was sluggishly motile. No sperms were seen in the control Fallopian tube. Sperms were rare in the operated tube, only about 5 per cent. of those seen being motile. The explanation of the rarity of spermatozoa in the cranial divisions of the genitalia appears to be that they had not yet gone forward from the cervix.

The sheep used for observation of the spermatozoa 12 hours after insemination was about 22 hours in oestrus at the time of service. The ovary had not yet ovulated at the time of slaughter.

No spermatozoa were seen in the vagina; some disintegrated remains were present. Sperms in the cervix were very numerous; about 40 per cent. were motile. In the pars indivisa of the uterus sperms were infrequent: about 58 per cent. were motile. In the control horn sperms were infrequent: about 70 per cent. were motile. Sperms were difficult to find in the operated horn; only two motile sperms were seen. In the normal tube spermatozoa were infrequent, but about 90 per cent. of those seen were motile. No motile sperms were present in the operated horn: a few non-motile intact sperms were present.

The sheep used for observation of the spermatozoa fifteen hours after insemination was about 13 hours in oestrus at the time of service. The ovary had not yet ovulated at the time of slaughter.

About 2 per cent. of the sperms seen in the vagina were sluggishly motile. Spermatozoa in the cervix were very numerous ; about 93 per cent. were motile. In the pars indivisa sperms were fairly numerous ; about 50 per cent. being motile. In the normal horn spermatozoa were fairly frequent : about 25 per cent. were motile. A few non-motile sperms were seen in the operated horn : no motile sperm was seen. Sperms were rare in the control tube ; only two motile sperms were seen. A few dead and disintegrated sperms only were seen in the operated tube.

The sheep used for observation of the spermatozoa eighteen hours after insemination was about 16 hours in oestrus at the time of service. The ovary had not yet ovulated at the time of slaughter.

Disintegrated sperms were seen in the vagina : one very sluggishly motile sperm was seen. Spermatozoa were numerous in the cervix ; about 47 per cent. were motile. There was little difference in the frequency of the sperms in the control and operated horn ; motility was sluggish in those seen which were still alive. In the control horn sperms were infrequent : only one of those seen was motile. Sperms were infrequent and mostly non-motile in the operated horn ; one motile sperm only was seen. Some non-motile sperms were seen in the normal tube. Only disintegrated remains were present in the operated tube.

The sheep used for observation of the spermatozoa twenty-four hours after insemination was about 14 hours in oestrus at the time of service. The ovary had ovulated at the time of slaughter. No spermatozoa were present in the vagina or in the divisions of the genitalia above the cervix. In the operated horn and tube there was no trace of spermatozoa. Sperms were fairly frequent in the cervix ; about 10 per cent. showing motility.

A control operation was performed on sheep 0·312, Table I, to ascertain if anaesthesia and puncture with insertion of the pipette to the tube and horn without ligature would have any detrimental effect on the genitalia and consequent unfavourable influence on the spermatozoa. The sheep used for this observation was about 17 hours in oestrus at the time of insemination. The ovary had not yet ovulated at the time of slaughter 18 hours later.

There was one motile sperm seen in the vagina. Sperms were frequent in the cervix : 70 per cent. being motile. In the pars indivisa the sperms were not nearly so frequent as in the cervix ; about 40 per cent. were motile. The spermatozoa in the genitalia cranial to the pars indivisa were equally numerous on the operated and control sides ; just under 50 per cent. showing motility.

Table I shows in summarised form the results of the observations :—

Table II shows the breeding records of the three rams used for obtaining spermatozoa :—

TABLE I.

Ewe No.	Period in oestrus when served.	No. of services.	Rams used.	Time elapsed since service and operation.	How Examined.	Compartment of Genitalia Examined.						
						Vagina.	Cervix.	Uterus, pars indivisa.	Uterus.		Fallopian Tubes.	
									Normal Horn.	Operated Horn.	Normal.	Operated.
0·311..	±18 hrs.	3	W. 31 x 1 T. 413 x 1 T. 417 x 1	6 hours (not ovulated).	F.	+(10%)	+++(55%)	+++(71%)	+++(68%)	++(44%)	++(40%.)	++(21%)
					S.	$+^1$	$+^1$	$+^1$	$+^1$	$+^1$	$+^1$	$+^1$
0·83...	± 9 hrs.	3	W. 31 x 1 T. 413 x 1 T. 417 x 1	9 hours (not ovulated).	F.	+++(50%)	++++(90%)	Only few sperms, some motile.	Sperms very rare, no motile sperm seen.	Sperms very rare, two intact, one non-motile.	—	+(5%)
					S.	$+^1$	$+^1$	$+^1$	$+^1$	$+^1$	$+^1$	$+^1$
0·259..	±22 hrs.	3	T. 413 x 1 T. 417 x 2	12 hours (not ovulated).	F.	0	++(40%)	+++(58%)	+++(70%)	Sperms very rare, two seen.	++++(90%)	0
					S.	—	$+^1$	$+^1$	$+^1$	$+^1$	$+^1$	—
0·239..	±13 hrs.	3	W. 31 x 1 T. 413 x 1 T. 417 x 1	15 hours (not ovulated).	F.	+(2%)	++++(93%)	+++(50%)	++(25%)	0	Very rare, two seen.	0
					S.	$+^1$	$+^1$	$+^1$	$+^1$	$+^1$	$+^1$	$+^1$ (disintegrated remnants).
0·96...	±16 hrs.	3	W. 31 x 1 T. 431 x 1 T. 417 x 1	18 hours (not ovulated).	F.	One sperm showing slight motility.	++(17%)	Sperms very rare, few sluggishly motile.	One motile sperm seen.	One motile sperm seen.	0	0 (disintegrated remnants).
					S.	$+^1$	$+^1$	$+^1$	$+^1$	$+^1$	$+^1$	—
0·292..	±14 hrs.	3	W. 31 x 2 T. 417 x 1	24 hours (ovulated).	F.	—	+	—	—	—	—	—
					S.	—	$+^1$	—	—	—	—	—
0·312..	±17 hrs.	2	T. 413 x 1 T. 417 x 1	18 hours (not ovulated).	F.	—	+++	++	++	+	++	++
					S.	—	$+^1$	$+^1$	$+^1$	$+^1$	$+^1$	$+^1$

++++ = 75 to 100 per cent. motile.
+++ = 50 to 75 per cent. motile.
++ = 25 to 50 per cent. motile.

+ = 1 to 25 per cent. motile.
0 = Non-motile sperms only.
— = No sperm seen.

$+^1$ = Sperms seen in stained preparations.
F. = Fresh preparations.
S. = Stained preparations.

TABLE II

Ram No	First Service				Second Service				Third Service				All Services			
	Number Served	Number Fertilised	Number not Fertilised	Percentage Fertilised	Number Served	Number Fertilised	Number not Fertilised	Percentage Fertilised	Number Served	Number Fertilised	Number not Fertilised	Percentage Fertilised	Number Served	Number Fertilised	Number not Fertilised	Percentage Fertilised
T 413	26	22	4	84.6	2	2	—	100	—	—	—	—	26	24	2	92.3
T 417	23	19	4	82.6	2	1	1	50	—	—	—	—	23	20	3	87.0
W 31	25	19	6	76.0	6	5	1	83.3	1	1	—	100	25	25	—	100.0
TOTAL	74	60	14	81.0	10	8	2	77.7	1	1	—	100	74	69	5	93.1

DISCUSSION.

From the literature available of work done on laboratory animals, namely the rabbit, guinea pig, and the mouse it appears that non-specific genital secretions have a definite detrimental influence on the life of spermatozoa [Yochem (1929)]. Further, it appears that admixture with female genital secretions curtails the duration of fertilising vitality, and the duration of motility of the sperm as compared with contact with the male secretion [Hammond and Asdell (1926); Walton, Hammond and Asdell (1928)].

The female genital secretions are more favourable to spermatozoa during oestrus than during the interoestrous period. The unfavourable influence of the secretions of the vagina in comparison with the secretions of the uterus on the motility of sperms has been noted by Löw (1902), in the case of the mouse and Hutschenreiter (1915) in the case of the mare. Kugota (1929) has shown that the secretion of the uterus has a varying influence on the spermatozoa at different periods during the oestrous cycle.

There appears to be no doubt, in the case of sheep, that the sperm does not as a rule survive contact with vaginal secretion for more than 12 hours. Occasionally isolated motile sperms may be seen up to 18 hours or even 24 hours following coitus, but this is exceptional. Spermatozoa in the cervix survive up to 48 hours. This indicates that there is a different influence in the different compartments [Quinlan, Maré and Roux (1932)]; in óne division it is more favourable to spermatozoon life than in the others.

In the case of the human species the summary of Giles (1919) indicates that sperms survive longer in the cervix than in the uterus and vagina.

The literature is scanty in reference to the influence of genital secretions on spermatozoa when their survival in the different divisions of the reproductive tract is studied. The point is, however, not without practical importance in breeding.

If, as the authors suggest, the cervix is the natural habitat of the spermatozoa in the ewe, while awaiting the arrival of an available ovum, a healthy condition of this portion of the reproductive tract is of the utmost importance in conception. Cervicitis does not appear to be a common condition in sheep, but its incidence in cattle is frequent. The uterine cervices of the bovine and the ovine have an anatomical similarity and it is highly probable that both perform similar physiological functions in relation to the spermatozoa.

It is evident from the previous work of Quinlan, Maré and Roux (1932) that the vagina is not the natural habitat of the spermatozoa of the ewe after copulation. They lose motility within a few hours in this part of the genital passage. The vagina appears to act only as a portal of entrance to the ostium uterinum.

The cervix would appear to be the portion of the female reproductive tract physiologically adapted to act as the natural habitat of spermatozoa while awaiting the arrival in the Fallopian tube of an ovum available for impregnation. Its secretion appears to be highly favourable to the life of the sperms. They remain numerous and active in this division even up to 48 hours after copulation.

It appears, in view of the relative infrequency of spermatozoa in the pars indivisa of the uterus, the uterine horns, and the Fallopian tubes, that the cervical reservoir is called upon for a constant small supply of sperms as long as any survive there. From the observations made in a very large number of ewes there appears to be no swarming forward of sperms to the tubes following copulation.

The results of the present series of experiments indicate that sperms which become located in the uterus, uterine horns, and the Fallopian tubes do not survive longer than 10 to 12 hours in these situations. If the uterine horns and tubes were the natural habitat of sperms why do not those placed there and isolated remain alive for longer than 10 to 12 hours?

So far as one can see from the operation there results no pathological change in the genital tract to account for the rapid death of spermatozoa transferred to the Fallopian tube and uterine horn, compared with those from the same ejaculation which become located in the cervix.

It is maintained that the few live sperms which were found in the operated horn after the 12th hour are not any remaining from those injected into its apex, but rather some which have come forward from the cervix, similar to the condition prevailing on the non-operated, control side. That the operation of manipulation and injection is harmless is definitely proved in the case of the control experiment on sheep 0·312, Table I, where the conditions in both sides of the genitalia were similar at slaughter, 18 hours after insemination.

CONCLUSIONS.

1. A study has been made of the motility of the spermatozoa of three highly fertile rams in the different divisions of the reproductive tract of normal Merino ewes.

2. The end-point of motility of spermatozoa in the vagina appears to be about 12 hours. The majority have ceased to be motile before the 12th hour; very occasional sluggishly motile sperms are present up to 18 and even 24 hours.

3. Spermatozoa may be numerous and actively motile in the cervical canal 24 hours after copulation.

[The present series of experiments extended only to the 24th hour, but previous experiments carried out by the authors (1932) have shown that living spermatozoa may be found in the cervix 48 hours after copulation.]

4. Spermatozoa injected into the lumen of the apex of the uterine horn do not survive contact with the uterine secretion for more than 12 hours. In fact the end-point of motility appears to be about the 9th hour.

5. Spermatozoa injected into the isolated Fallopian tube through its ovarian opening do not retain motility for more than a few hours; .21 per cent. were motile after 6 hours; 5 per cent. after 9 hours; no motile sperms were seen after 12 hours.

6. The secretion of the vagina is unfavourable to the motility of spermatozoa.

7. The secretion of the cervix is more favourable than that of the other divisions of the genitalia to the life of spermatozoa.

8. The secretion of the uterus and Fallopian tubes is unfavourable to spermatozoa artificially transferred to these situations without passage through the cervix.

9. The cervical canal appears to be the natural habitat of spermatozoa while awaiting the arrival of an available ovum; small numbers of sperms are constantly passing forward through the uterus to the uterine horns and the Fallopian tubes. Under favourable conditions the cervix acts as a depôt for spermatozoa from which there is a constant issue of activity motile sperms to the cranial divisions of the reproductive tract.

10. The injury to the Fallopian tube and uterine horn by the operation did not prevent sperms from acting normally, as shown by a control operation following normal copulation.

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Onderstepoort Journal of Veterinary Science and Animal Industry, Volume I, Number 1, 1933.

Plant Poisoning in Stock and the Development of Tolerance.

By D. G. STEYN, B.Sc., Dr. Med. Vet., Veterinary Research Officer, Onderstepoort.

INTRODUCTION.

A.—GENERAL.

As it was found (Steyn, 1932) that it was possible to cause the development of tolerance to *Chrysocoma tenuifolia* Berg. poisoning in goats by repeatedly drenching these animals with small amounts of this plant, it was decided to ascertain whether such a tolerance would also be developed in poisoning by other plants.

It is a well known fact that an active and specific immunity can be produced against those plants containing toxalbumins as active principles. These toxalbumins are abrin (*Abrus precatorius* Linn.), modeccin (*Adenia digitata* Engl.), crotin (*Croton tiglium Linn.*), curcin (*Jatropha curcas* Linn.), ricin (*Ricinus communis* Linn.), and robin (*Robinia pseudacacia* Linn.).

This immunity, which must be distinguished from tolerance, may be developed to such an extent that an animal repeatedly treated with non-toxic amounts of the above toxalbumins may tolerate, without any apparent ill-effects, up to eight hundred times the minimum lethal dose. This highly developed immunity is due not to an habituation of the tissues to the poison but to the development of specific antitoxins in the serum.

Much progress has lately been made in the immunisation of human beings against the pollens of some plants (hay fever), a problem to which many references are to be found in the literature.

B.—HISTORICAL.

Schamberg (1919) produced a tolerance to "*Rhus toxicodendron*" in human beings by giving per os small and increasing doses of the tincture to susceptible persons. Strickler, Schamberg's assistant, succeeded in preventing attacks of dermatitis in human beings caused by this plant by injecting them subcutaneously with an alcoholic extract of the plant. Schamberg has found that the "immunity" set up by his method generally does not persist longer than one month after the discontinuation of the administration of the tincture.

Sutton (1919) discusses the relation between anaphylaxis and immunity and, quoting Cooke, says that when few antibodies or none are present, the non-sensitive state exists; when antibodies are numerous and attached to the body cells, the sensitive or anaphylactic state prevails; and when antibodies are in excess, with many unattached to body cells, the immune state prevails. He states that "anaphylaxis and immunity are the same in principle differing only quantitatively."

Ratner and Gruehl (1927-1928) demonstrated that normal guinea pigs when exposed to an organic dust (horse dander) could become sensitised through inhalation. Guinea pigs thus sensitised and subsequently exposed to the same dust after a suitable incubation period, exhibited unmistakable signs of anaphylaxis, which the authors term "respiratory anaphylaxis." Further experiments proved that typical respiratory anaphylaxis (bronchial asthma) can be produced in guinea pigs by allowing them to inhale castor bean dust and again exposing them to this dust after an incubation period of two to three weeks.

Figley and Elrod (1928) refer to the occurrence of a large number of cases of asthma caused by the inhalation of castor bean dust liberated in the air from the pipes of a castor oil factory.

Petri (1930) mentions that a condition known as "fabismus" arises when the fruit of "Vicia faba" is eaten or when its pollen is inhaled. This condition, which is characterised by a rapid development of anaemia. icterus with urobilinuria, and swelling of the spleen and liver, is supposed to be an "intolerance" to "Vicia faba." Petri expresses no definite opinion as to whether this condition is due to direct poisoning or is an anaphylactic phenomenon.

Bürgi (1931) states that a tolerence to *Taxus baccata* (Yew) can be produced in horses by feeding them small amounts of the plant.

Mackay (1931) was able to produce in rats a tolerance to morphine by administering this drug per os and found an increase of 70 per cent. in the weight of the adrenal glands in such morphine treated rats. Most of this increase had occurred in the cortex of the adrenals.

Tatum and Seevers (1931) made a valuable contribution to the study of drug addiction. They define addiction, tolerance and habituation as follows: "Addiction is a condition developed through the effects of repeated actions of a drug such that its use becomes necessary and cessation of its action causes mental or physical disturbances."

"Tolerance is a condition developed by certain drugs such that progressively larger and larger quantities are required to produce the effects desired."

"Habituation is a condition in which the habitue desires a drug but suffers no ill effects on its discontinuance."

Some drugs produce addiction and no tolerance (cocaine) and vice versa (organic nitrites) while others produce both (morphine).

With regard to strychnine and cocaine Tatum and Seevers state that experiments on animals point to increased sensitivity rather than tolerance.

Biggam, Arara and Ragab (1932) refer to drug-addiction in Egypt in which heroin, opium, morphine, háshish, manzoul, cocaine and mixtures of these drugs are concerned. The withdrawal symptoms exhibited by these addicts are restlessness, sleeplessness, excitability, irritability, sneezing, yawning, lachrymation, colic, diarrhoea, headaches, vomiting, and pains in the limbs. These symptoms persist for about four days and then subside. They have found that a substitution therapy with atropine, morphine, strychnine, paraldehyde, luminal and magnesium sulphate relieves the withdrawal symptoms very markedly.

Santesson (1932) succeeded in producing a tolerance in rabbits to copper sulphate by injecting them subcutaneously with small and increasing quantities of this salt.

Simpson and Banerjee (1932) state that horses develop a tolerance to *Abrus precatorius* when the seeds are given in small and gradually increasing doses.

Speight (1932) states that ill-health and insanity are inevitable results of the excessive and continued use of dagga (*Cannabis sativa*).

ONDERSTEPOORT EXPERIMENTS.

ASCLEPIADACEAE.

Asclepias physocarpa Schltr.

Registered number: Onderstepoort Spec. No. 5333; 7/1/32.

Common name: Melkbos; wild cotton; milkweed.

Origin: Entembeni, Hluhluwe, Zululand.

State and stage of development of plant: Dry and in late flowering and seeding stage.

The results of experiments to determine the toxicity of this plant and to ascertain whether animals are liable to develop a tolerance when repeated and increased amounts of this plant are ingested are recorded in the following table:—

TABLE I.

EXPERIMENTS WITH *ASCLEPIAS PHYSOCARPUS* SCHLTR. ON SHEEP.

D.O.B. No.	Quantity of plant given and dates of dosage.	Total amount of plant given. gm.	Period of dosage.	Result.
28203	100 gm. on 13/1/32	100	1 dose only	Within two hours after dosage dyspnoea and an accelerated pulse set in. In the course of the next two days cyanosis, hoven, groaning, pronounced dyspnoea, a weak and accelerated pulse, fever, inappetence, apathy and a pronounced foetid diarrhoea were present. Improvement set in on the third day, the animal being in normal health again on 25/1/32.
26446	300 gm. on 12/1/32	300	1 dose only	Symptoms set in within one hour after dosage—apathy, inappetence, cyanosis, accelerated pulse, dyspnoea, fever, the animal dying with symptoms of asphyxia seven hours after dosage. *Post-mortem appearances:* General cyanosis, pronounced hyperaemia of the lungs and spleen, localised hyperaemia of abomasum, slight acute catarrhal duodenitis and caseous lymphadenitis (bronchial lymph glands).

TABLE I—(*continued*).

D.O.B. No.	Quantity of plant given and dates of dosage.	Total amount of plant given. gm.	Period of dosage.	Result.
32313	20 gm. daily * from 16/1/32–19/1/32	80	4 days....	Diarrhoea with its accompanying symptoms set in on 19/1/32. 20/1/32—profuse diarrhoa; animal appears very ill. Dosing discontinued. Treated with a mixture of carron oil ... 1·0 gm. of tannic acid. 25/1/32—appears to be in normal health.
31578	20 gm. daily from 14/1/32–17/1/32 10 gm. daily from 18/1/32–1/2/32 20 gm. daily from 2/2/32–7/2/32 30 gm. daily from 8/2/32–15/2/32 40 gm. daily from 16/2/32–28/2/32 70 gm. daily from 29/2/32–6/3/32 80 gm. on 7/3/32.	1510	54 days...	17/1/32—inappetence and dyspnoea; hence daily dose reduced to 10 gm. 7/3/32—within four hours after dosing, diarrhoea and symptoms similar to those described above appeared; death occurring at 4 p.m. *Post-mortem appearances:* Pronounced general cyanosis, pronounced hyperaemia of lungs; slight hyperaemia of abomasum; slight acute catarrhal duodenitis and jejunitis; pronounced acute catarrhal colitis with haemorrhages in the mucosa; oesophagostomiasis (nodular form).
31485	5 gm. daily* from 20/1/32–1/2/32 10 gm. daily from 2/2/32–7/2/32 20 gm. daily from 8/2/32–14/2/32 30 gm. daily from 15/2/32–21/2/32 50 gm. daily from 22/2/32–24/2/32 Not dosed from 25/2/32–6/3/32 50 gm. daily from 7/3/32–13/3/32 60 gm. daily from 14/3/32–17/3/32	1105	58 days...	25/2/32—pronounced diarrhoea accompanied by inappetence, apathy, dyspnoea and an accelerated and weak pulse. Treated with carron oil + 1·0 gm. of tannic acid. 7/3/32—apparently healthy. 18/3/32—animal appears very ill—pronounced foetid diarrhoea and fever. 28/3/32—apparently healthy.

* Except Sundays.

From the above table it would appear that *Asclepias physocarpa* Schltr. is a severe gastro-intestinal irritant and that sheep are not likely to develop a tolerance when this plant is taken repeatedly in small amounts. On the contrary, it appears that there is a tendency for the development of cumulative effects when non-toxic amounts of the plant are taken continuously.

COMPOSITAE.

Centaurea picris DC.

Registered number: Onderstepoort Spec. No. 4594; 8/12/31.

Common name:

Origin: On cultivated lands, Carolspoort, De Aar.

State and stage of development of plant: Dry and in flowering and early fruiting stage.

This plant is referred to in the article titled "Poisoning of Human Beings by Weeds contained in Cereals (bread poisoning)" appearing elsewhere in this report.

The following table reflects the results obtained in an attempt to produce a tolerance to this plant in sheep:—

TABLE II.

EXPERIMENTS WITH *CENTAUREA PICRIS*. DC. ON SHEEP.

D.O.B. No.	Quantity of plant given and dates of dosage.	Total amount of plant given. gm.	Period of dosage.	Result.
Merino Sheep 31943 (full mouth)	8/12/31—600 gm. (in two doses of 300 gm. each)	600	One day...	8/12/31. Symptoms appeared within two hours after the second dose. Pronounced dyspnoea, hoven, groaning, weak and accelerated pulse, apathetic and fever. Died within twenty hours after first dose. *Post-mortem appearances:* General cyanosis; heart in systole; marked hyperaemia of lungs; nodular oesophagostomiasis.
Merino Sheep 31825 (full mouth)	9/12/31—300 gm., 10/12/31—300 gm., at 8.30 a.m., 300 gm. at 2 p.m.	900	Two days.	Symptoms appeared at 3 p.m. on 10/12/31 and were similar to those described in sheep 31943. Death occurred within thirty-six hours after the first dose. *Post-mortem appearance:* Intense general cyanosis; hydroperitoneum; hydrothorax; hydropericardium; pronounced hyperaemia and slight oedema of the lungs; acute catarrhal gastro-enteritis with numerous haemorrhages in mucosa of of small intestine; oesophagostomiasis.

TABLE II—(*continued*).

D.O.B. No.	Quantity of plant given and dates of dosage.	Total amount of plant given. gm.	Period of dosage.	Results.
Merino Sheep 31578 full mouth)	300 gm. daily* from 11/12/31 to 17/12/31. 300 gm. daily from 18/12/31 to 22/12/31. (23/12/31—300 gm. 9 a.m. and 300 gm. 2 p.m. 24/12/31 —300 gm. at 9 a.m. 300 gm. at 2 p.m.)—Tolerance test.	3700	14 days...	15/12/31. Apathetic; inappetence, fever, dyspnoea, strong and accelerated pulse. Not dosed. 16/12/31.—Appeared healthy. 23/12/31–24/12/31 : Tolerance test. No ill-effects were noticeable.
Merino Sheep 28895 (full mouth)	28/12/31—300 gm. at 8.30 a.m., 300 gm. at 2 p.m.	600	One day...	Died within twenty hours after the first dose with symptoms similar to the above. *Post-mortem appearances :* General cyanosis; congestion of subcutaneous tissues; hydroperitoneum; hydrothorax; dilatation of both heart ventricles; pronounced hyperaemia and slight oedema of lungs; pronounced hyperaemia of and haemorrhage in the bronchial, mediastinal and retrophyrangeal lymph glands; marked acute catarrhal gastro-enteritis.
Merino Sheep 31485 (full mouth)	100 gm. daily from 29/12/31–2/1/32. 200 gm. daily from 4/1/32 to 9/1/32. (11/1/32–300 gm. at 8.30 a.m., and 300 gm. at 2 p.m. 12/1/32–300 gm. at 8.30 a.m., 300 gm. at 2 p.m.)—Tolerance test.	2900	15 days...	This animal developed no symptoms of poisoning.

* Except Sundays.

The above results tend to show that small and increased amounts of *Centaurea picris* DC. are liable to produce a tolerance in sheep, in as much as a dose of 600 grams of the dry plant caused death in susceptible sheep, whilst it produced no ill-effects in sheep which had been subjected to a preliminary treatment with non-toxic amounts of the plant.

The fact that 600 grams of the dry plant caused death in sheep 28895 on 28/12/31 is proof that the plant has not decreased in toxicity on storage.

Muir (1928) found *Centaurea melitensis* on wheatlands in the Riversdale area. No records of its toxicity could be found in the available literature.

DISCUSSION.

Tolerance and immunity must be distinguished from each other as they are used to describe two completely different phenomena as far as desensitisation to plant poisons is concerned.

Tolerance means an increase in resistance to plant poisons of a non-albuminoid nature. The nature of this resistance is still an unsettled problem. This state of desensitisation is due probably to a mobilisation of the defensive powers of the system and the following hypotheses may be advanced with regard to the development of acquired tolerance to poisons :--

(a) *Cellular immunity.*—When living cells are brought into contact with low but increasing concentration of poisons, it is possible that these cells will in the course of time adapt themselves to their new environment and perform their functions in a normal way in spite of the fact that they are bathed in a fluid which under normal circumstances would have seriously interfered with their activities. To elucidate this point I might mention drug-fast bacteria and protozoa. This cellular immunity may be intracellular or extracellular or both. That is, the cells may allow the poison to enter into their interior and they may in some or other way inactivate or destroy the poison within their structure ; or, they may develop their defensive powers to such an extent as not to allow the poison to enter into their protoplasm. The latter process may be termed "selective osmosis."

(b) *Increased inactivation and (or) augmented rate of destruction of the poison.*—It would appear possible that the activities of the body tissues and the liver, as detoxicators, and of the organs of excretion (liver, kidneys, skin, gastro-intestinal mucosa, lungs, lactating glands) may be increased when sufficient time is available in order to allow of the development of such an increase in activity by gradually introducing into the system non-toxic and increasing amounts of a poison.

It is a most interesting phenomenon that a tolerance is developed to one poison whilst another will cause a hypersensitivity when taken in repeated small amounts. Of still greater interest is the fact that a certain organ may develop a tolerance to a certain poison, whereas another organ will become sensitised to the same poison. As an example of the latter type of poison caffeine, which causes desensitisation of the kidney and sensitisation of the central nervous system, may be quoted.

According to Tatum and Seevers (1931) those drugs, which decrease the activity of cells, tend to cause the development of tolerance, whilst those which stimulate the activity of cells, tend to produce an increased sensitivity.

Animals, which have developed a tolerance to some or other poison (acquired tolerance) can hardly be regarded as normal as some or other physiological or (and) histological change is bound to have occurred in their system. Desensitisation may in many cases be explained by a cumulative action of that particular poison, as is, for example, the case in repeated doses of strychnine and digitalis.

Contrary to tolerance, immunity to certain plant poisons (toxalbumins) is a definitely conceived phenomenon, in that, by reason of their albuminoid nature, they cause the production of antibodies in the body tissues.

Tolerance was produced in sheep to *Centaurea picris* DC. whilst *Asclepias physocarpa* Schltr. appeared to cause the development of an increased sensitivity.

SUMMARY.

(1) It is possible to cause the development of tolerance in animals, to certain poisonous plants, by drenching them with small and increasing quantities, whilst others do not produce this phenomenon and may even cause sensitisation, or have cumulative effects.

(2) The well known fact that animals, newly introduced to farms infested with poisonous plants, are much more liable to succumb to plant poisoning than animals born and reared on such farms, is most probably partly due to an acquired tolerance developed in the course of time by repeatedly partaking of small quantities of these plants. It is fully realised that discriminate feeding, which is a characteristic of stock reared in areas infested with poisonous plants, plays a very important rôle in the prevention of plant poisoning in these animals.

(3) Theories of tolerance and desensitisation are discussed.

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Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 1, Number 1, 1933.

The Toxicity of Sodium Chlorate.

By D. G. STEYN, B.Sc., Dr.Med.Vet., Veterinary Research Officer, Onderstepoort.

INTRODUCTION.

Very favourable results with regard to sodium chlorate as a weed-killer have been reported from New Zealand [Editorial (1930) and Lyons (1930)] and the United States of America [Editorial (1931)]. In New Zealand sodium chlorate has been officially recommended in a 4 to 5 per cent. aqueous solution as an efficient killer of ragwort (*Senecio spp.*), whilst in the United States of America it is advocated as a general weed-killer, being especially destructive to thistles and many graminaceous weeds. The spraying of weeds during early stages of development is advised as they are less resistant than fullgrown plants and also less spraying material is required.

In New Zealand the effects of calcium chlorate as a weed-killer have been compared with those of sodium chlorate and the latter was found to be cheaper and more effective than the former.

Since *Senecio* poisoning is of very widespread occurrence in the Union of South Africa, it has been decided to conduct some experiments in order to test the value of sodium chlorate as a weed-killer under South African conditions with special regard to *Senecio spp.* and other poisonous plants (*Dichapetalum cymosum* Hook, *Pachystigma pygmaeum* Robyns, etc.).

The toxicity of such a substance to stock is of the utmost importance in the determination of its value as a weed-killer. This statement needs no lengthy elucidation, as it is wellknown that some arsenical preparations are excellent weed-killers but are at the same time a grave danger not only to stock but also to the persons handling such poisonous preparations. It might be mentioned here that the general practice of eradicating prickly pear by means of arsenic pentoxide has been responsible for serious losses in stock.

It is clear that an ideal weed-killer should be highly toxic to the weed or weeds to be destroyed and relatively non-toxic to edible and useful plants and to stock and human beings.

According to reports from New Zealand and the United States of America, sodium chlorate seems to fulfil these requirements better than any known weed-killers.

It was thought advisable to ascertain the toxicity of sodium chlorate under South African conditions before recommending it as a general weed-killer.

REVIEW OF LITERATURE.

Fröhner (1919) administered 25 grams of potassium chlorate to a 35 Kg. wether with negative results, whilst 50 grams caused transient depression, inappetence and cessation of rumination. He found that amounts of 30 and 40 grams had no effect on horses and that a cow, which had received 50 grams, and two days later, 100 grams of potassium chlorate, developed no symptoms of poisoning.

Zimmerman (quoted by Fröhner 1919) states the following to be the lethal doses of potassium chlorate: horse 250 grams; cow 500 grams; sheep 100 grams; dogs 60 grams.

Seddon and Mcgrath (1930) fed a lick containing two parts of sodium chlorate and one part of bonemeal to a bovine. On the fourteenth day of the experiment after it had ingested 260 grams of sodium chlorate the animal exhibited inappetence and depression, the faeces being dark and mucoid. An intense icterus developed and the animal was destroyed on the tenth day of illness.

Seddon and Mcgrath found the toxic dose of sodium chlorate for sheep to be from 50–75 grams. Thirty-three grams of this salt had no effect on sheep whilst one sheep died from 50 grams and another one developed no symptoms after the administration of the latter amount.

Lipschitz (1932) fed cats for weeks on a diet to which 0·5 gram of sodium and potassium chlorate per kilogram body weight had been added daily without producing any symptoms of poisoning. A single dose of 1·13 grams potassium and sodium chlorate per kilogram body weight caused slight symptoms, whilst 1·35 to 1·94 grams of sodium chlorate per kilogram body weight produced pronounced dyspnoea, methaemoglobinaemia and death in cats.

SYMPTOMS OF POISONING.

(*a*) *Acute poisoning.*—Large amounts of sodium and potassium chlorate cause death one to a few hours after administration, the proximate cause of death being asphyxia due to severe methaemoglobinaemia which was diagnosed spectroscopically. There is also a certain degree of haemolysis.

On post-mortem examination the blood and organs (especially the lungs) are found to be dark chocolate brown in colour. If the blood be centrifuged the supernatant serum is of a reddish tinge due to haemolysis.

(*b*) *Subacute poisoning.*—When small amounts of the chlorates are repeatedly administered the following symptoms may be seen: laboured respiration; accelerated heart action, which becomes progressively weaker; gastro-intestinal irritation; inappetence; cessation of rumination; intense icterus (haematogenic); haemoglobinaemia; haemoglobinuria; anuria; uraemia; opisthotonus; uraemic spasms; disappearance of the petallar and corneal reflexes; coma and death.

The *post-mortem* reveals icterus of a varying degree depending on the period of illness; enlarged and copper coloured liver; pigmented and swollen kidneys; gastro-enteritis and cystitis.

On pastures treated with sodium chlorate the subacute and chronic forms of poisoning with this weed-killer are more likely to occur than the peracute and acute forms.

ONDERSTEPOORT EXPERIMENTS.

The experiments conducted at Onderstepoort are recorded in the following table. Commercial sodium chlorate was administered per stomach tube in a 10 per cent. aqueous solution. The commercial preparation was used as it is cheaper than chemically pure sodium chlorate and will, therefore, be used in preference to the latter as a weed-killer.

THE EFFECTS OF SODIUM CHLORATE ON HORSES, SHEEP AND RABBITS.

D.O.B. No. of animal.	Weight in Kg.	Quantity of NaClO^3 given, and period of dosage.	Total amount of NaClO^3 given.	Result.
			Gm.	
Rabbit A.	2·0	1 gm. daily* for 20 days; 3 gm. daily for 6 days; 15 gm. in one dose	53	The 1 gm. doses continued for 20 days had no effect; likewise the 3 gm. doses over a period of 6 days. 15 gm. caused dyspnoea 1 hour after dosage; then pronounced apathy; weak and accelerated heart beat; brownish conjunctiva; passage of dark brown urine; fever; weakness and death within 10 hours after dosage. *Post-mortem appearances:* Blood and all organs (especially the lungs) of an intense dark chocolate brown colour; haemolysis; haemoglobinuria.
Rabbit B.	2·2	3 gm. daily for 20 days; 6 gm. daily for 3 days; 10 gm. daily for 2 days; 12 gm. in one dose	110	The 3 gm. doses over a period of 20 days had no effect; likewise the three 6 gm. doses. The 10 gm. doses produced symptoms as described above and diarrhoea. The 12 gm. dose caused death after 8 hours. *Post-mortem appearances:* As in Rabbit A plus acute catarrhal gastro-enteritis.
Rabbit C.	2·0	5 gm. daily for 20 days; 7·5 gm. daily for 3 days	122·5	The twenty 5 gm. doses caused no ill-effects. The third dose of 7·5 gm. caused symptoms as in Rabbit A and death 6 hours after administration. *Post-mortem appearances:* Pronouned hydraemia; blood of a slight brownish tinge; hyperaemia of the lungs and liver.
Rabbit D.	2·3	7·5 gm. in one dose; 10 gm. in one dose; 12 gm. in one dose	29·5	A few hours after the 7·5 gm. dose the animal passed dark brown urine and showed laboured respiration; accelerated and weak heart beat. More severe symptoms were produced by the 10·0 gm. dose. The animal died 55 hours after the 12·0 gm. dose after having exhibited apathy, pronounced weakness, pronounced dyspnoea, accelerated and weak heart action. *Post-mortem appearances:* Cachexia; pronounced hydraemia; extreme dilatation of atria and ventricles of heart.

* Except Sundays.

THE EFFECTS OF SODIUM CHLORATE ON HORSES, SHEEP AND RABBITS (*continued*).

D.O.B. No. of animal.	Weight in Kg.	Quantity of NaC10^3 given, and period of dosage.	Total amount of NaC10^3 given.	Result.
			Gm.	
Rabbit E.	2·1	10 gm. in one dose	10	Symptoms similar to those described in Rabbit A produced by the 10 gm. dose were exhibited, death occurring one hour after dosage. *Post-mortem appearances :* Blood and organs of an intense dark chocolate brown colour ; haemolysis.
Rabbit F.	2·2	10 gm. in one dose	10	Developed severe symptoms of poisoning but recovered.
Sheep 32313 (full mouth)	40·0	15 gm. daily for 3 days	45	The first two doses had no discernible effects. Three hours after the third dose the animal exhibited dyspnoea; accelerated heart beat, which became progressively weaker ; haemoglobinmaemia, temperature 160° F. ; visible mucous membranes ; conjunctiva and unwoolled parts of the skin dirty brown in colour ; the animal died 20 hours after the third dose. *Post-mortem appearances :* Blood and organs (especially lungs) of an intense dark chocolate brown colour : haemoglobinaemia ; haemoglobinuria ; pronounced oedema and hyperaemia of lungs ; oedema of all lymph glands ; marked tumor splenis ; degenerative changes in the liver ; subendocardial haemorrhages ; numerous haemorrhages in mucous membrane of caecum and colon.
Sheep 28203 (full mouth)	45	30 gm. daily for 2 days	60	The first dose of 30 gm. caused slight dyspnoea and accelerated heart beat which passed off a few hours. The second dose produced the same train of symptoms as that described in sheep 32313 (temp. 108° F.) death following within 20 hours of the record dose. *Post-mortem appearances :* As in sheep 32313.
Sheep 31599	35	7·5 gm. daily for 20 days ; 10 gm. daily for 3 days	180	After the second dose of 7·5 gm. petechiae were noticed on the conjunctiva and disappeared within 24 hours. Inappetence and diarrhoea set in on the twelfth day of dosage ; complete recovery, however, had occurred on the seventeenth day of the experiment in spite of the fact that daily dosage was continued. The three 10 gm. doses produced no clinical symptoms.

THE EFFECTS OF SODIUM CHLORATE ON HORSES, SHEEP AND RABBITS (*continued*).

D.O.B. No. of animal.	Weight in Kg.	Quantity of $NaClO^3$ given, and period of dosage.	Total amount of $NaClO^3$ given.	Result.
			Gm.	
Horse 18526 (aged)	450	30 gm. on 4/4/32 60 gm. on 11/4/32 90 gm. on 18/4/32 120 gm. on 2/5/32 130 gm. on 16/5/32	430	The doses of 30 and 60 gm. caused slight brownish discolouration of the conjunctiva and laboured respiration, recovery occurring after 36 hours. The 90 gm. dose produced dark dirty brown discolouration of the conjunctiva with ecchymoses and dyspnoea which persisted for about 2 days. The 120 and 130 gm. doses caused a pronounced discolouration of the conjunctiva; somnolence; inappetence; accelerated and strong pulse; temparature of 102° F.; dyspnoea. The animal appeared normal after a further two days.

From the above table it is evident that sodium chlorate given in daily amounts up to 5 grams in a 10 per cent. solution produced no appreciable ill-effects on rabbits, whilst 10 grams in a single dose sufficed to produce death one hour after administration in one rabbit, and on another rabbit severe symptoms of poisoning which, however, did not prove fatal.

In sheep three daily doses of 15 grams each produced death twenty hours after administration of the last dose, whilst two 30 gram doses sufficed to cause death. Again in the course of the administration of twenty daily doses of 7·5 grams transient diarrhoea and inappetence appeared, the animal recovering in spite of the fact that dosing was continued and the daily quantity increased to 10 grams for the last three days of dosage.

A horse developed fairly severe symptoms of poisoning after the administration of 120 and 130 grams of sodium chlorate respectively, whilst 60 grams produced only slight methaemoglobinaemia.

The proximate cause of death in acute sodium chlorate poisoning is asphyxia due to severe methaemoglobinaemia, death being accelerated by haemolysis.

In protracted cases methaemoglobinaemia becomes a less prominent symptom and may not be manifested clinically whilst hydraemia and uraemia enter into the symtom complex.

It must be mentioned that in cases of sodium chlorate poisoning no reference to elevation of body temperature is made in the literature, while in the cases produced at Onderstepoort high fevers were recorded in all animals, especially in severe cases of poisoning.

Dr. G. de Kock, head of the department of Pathology, Onderstepoort, who has kindly examined the organs of rabbits and sheep poisoned by sodium chlorate reports that the former animals showed hyperaemia of the different organs and fibrosis of the myocard. In the case of sheep all the organs were hyperaemic and the kidneys showed a peculiar pigmentation, which was not due to haemosiderin or haemoglobin.

DISCUSSION.

In discussing weed-killers the most important point that arises is the possibility of their causing poisoning in stock. It is obvious that weed-killers which are to be extensively used on pastures should not be so toxic as to cause poisoning in such amounts as are likely to be ingested with the vegetation. The toxicity of arsenical compounds precludes their use as weed-killers except in special cases, for example, in localised spots.

Another point of the utmost importance in the extensive application of weed-killers to pastures is the degree of damage they will cause to the edible and valuable vegetation in such solutions as will destroy the weeds. This relative destructive value of weed-killers to weeds and edible vegetation is perhaps of more value than the degree of toxicity of the weed-killers to stock, as the poisoning of stock could be prevented by not allowing them access to treated pastures until after heavy rains have fallen.

From the results of experiments conducted by Fröhner (1919), Seddon and Mcgrath (1930) and at Onderstepoort. it would appear that sodium chlorate is relatively speaking, not very toxic to stock. As reports from New Zealand and the United States of America record it to be an efficient weed-killer, sodium chlorate would best seem to satisfy the requirements of a suitable weed-killer to be utilised on pastures.

However, before its use as a general weed-killer on pastures can be advocated, it is essential to conduct experiments in order to determine its relative destructive capacity for the weed or weeds to be killed and for the pasture plants. It is on this property that the suitability of sodium chlorate as a weed-killer on pastures depends.

SUMMARY.

In New Zealand sodium chlorate is recommended as an efficient destroyer of ragwort, while reports from the United States of America state it to be effective as a general weed-killer.

The fact that sodium chlorate is of comparative low toxicity to stock would seem to warrant the conducting of experiments with a view to ascertaining its value as a general weed-killer on South African pastures.

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Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 1, Number 1, 1933.

Lathyrus Sativus L. (Chickling Vetch; Khesari; Indian Pea) as a Stock Food.

By D. G. STEYN, B.Sc., Dr.Med.Vet., Veterinary Research Officer, Onderstepoort.

INTRODUCTION.

ON 15/5/31, a letter, in which information was sought as to the suitability of *Lathyrus sativus* L. hay as cattle feed, was received at Onderstepoort from the Cotton Breeding Station, Barberton. Specimens of this plant were submitted at the same time.

Lathyrus sativus L. (National Herbarium No. 11766; Onderstepoort Specimen No. 1388, 5/6/31) was grown at Barberton and it was noticed that it was not attacked by the numerous pests which were prevalent on the other winter crops grown at the above station. This phenomenon was mainly responsible for the interest taken in *Lathyrus sativus* as a stock feed.

This plant is stated (see Review of Literature) to have been responsible for losses in stock in various parts of the world, but as the toxicity of the same plant grown in different localities varies to a considerable extent it was thought advisable to find out whether the plant grown under South African climatic and soil conditions was poisonous and, if so, to determine the degree of toxicity.

REVIEW OF LITERATURE.

Lathyrus sativus, L. cicera and *L. clymenum* are used as articles of diet in India and Algeria, and both the plant (hay) and peas are grown on a large scale in Canada, Southern Europe, Algeria, and India as food for cattle, horses and other stock. Many cases of poisoning in human beings and stock due to the ingestion of these peas in large amounts are on record.

Pammel (1911) mentions that *Lathyrus cicera*, *L. clymenum* and *L. sativus* are poisonous and that the active principles are unknown.

Long (1917) refers to *Lathyrus cicera, L. clymenum* and *L. sativus* as having caused poisoning in man, horses, cattle, sheep and pigs, particularly in horses. Many cases of poisoning have been recorded in the veterinary journals since 1885. In 1884 *Lathyrus sativus* caused death in nineteen out of thirty-five affected horses, which took ill through eating these peas at the rate of three to four ℔. per head per day. Long quotes another case where *Lathyrus sativus* caused poisoning in a hundred and twenty-three out of eight hundred horses. Few cases of *Lathyrus*-poisoning in other classes of stock are recorded. Pigeons are stated to become partly paralysed and unable to fly. When boiled the peas lose part of their toxicity as the toxic substance passes into the water, which may contain such amounts of poison as to cause death.

Fröhner (1919) mentions that the information with regard to the toxicity of *Lathyrus cicer* (*N.B.* not to be confused with *Lathyrus cicera*) is very contradictory. Apparently this plant, which has always been considered as a valuable food for human beings and stock, has sometimes been confused with *Lathyrus sativus* and hence the records of its toxicity.

Anderson, Howard and Simonsen (1925) thoroughly investigated the toxicity of *Lathyrus* in an attempt to clear up the contradictory statements as to its harmfulness to human beings and stock. They obtained *Lathyrus sativus* seeds from thirty localities and found the only contaminating seed to be that of *Vicia sativa L. var. angustifolia.* These seeds were sown and it was soon evident that the "khesari" from the various localities were not identical. Further investigations proved that in addition to *Vicia sativa* the following plants may be found as weeds on *Lathyrus* fields: *Lathyrus sphaericus* Retz, *Lathyrus aphaca* and *Vicia hirsuta* Koch. Both the feeding trials and chemical examination of these three weeds yielded negative results.

The experiments of Anderson, Howard and Simonsen with *Lathyrus sativus* gave negative results, whilst with *Vicia sativa* they produced symptoms of poisoning in ducks and monkeys resembling those ascribed to *Lathyrus sativus.* These authors state that their experiments indicate that "khesari" is harmless and that the danger of disease lies in its contamination with *Vicia sativa.* They suggest the prevention of lathyrism by sowing the lathyrus seeds in rows one foot apart so as to allow of proper weeding in the early stages of growth.

Clough (1925) gave a very useful historical summary of *Lathyrus* poisoning.

Lander (1926) states that *Lathyrus sativus, L. cicera* and *L. clymenum* are harmless in the early stages of growth and that the toxicity sets in from the time of the formation of seed, the seeds being the most dangerous part.

Stockman (1929) produced *Lathyrus* poisoning in monkeys by feeding them on steamed *Lathyrus* peas. Rabbits fed on steamed *Lathyrus* peas for five months developed no symptoms. All guinea pigs fed on the steamed peas died after eight to thirty-five days, the cause of death being inanition.

A. The Toxic Principle.

At the Imperial Institute (Editorial 1917) the seeds of several forms of *Lathyrus sativus* obtained from India, Cyprus and Canada have been chemically examined but no toxic constituents could be isolated.

Long (1917) remarks that Smith (Bernhard-Smith) gives the active principle of *Lathyrus* as prussic acid and the former rightly disagrees as the symptoms of *Lathyrus* poisoning by no means resemble those of prussic acid poisoning. Fröhner (1919) mentions that nothing definite is known about the chemical nature of *Lathyrus* poison, and that it probably is an alkaloidal substance. Byam and Archibald (1921) state that the causative principle is not known.

Bernhard-Smith (1923) states that the toxic principle of *Lathyrus cicera* and *L. clymenum* is prussic acid. This incorrect statement was referred to by Long.

Anderson, Howard and Simonsen (1925) mention that Stockman and Dilling extracted from the seeds of *Lathyrus sativus* small amounts of alkaloidal substances, which on subcutaneous inoculation into animals produced symptoms similar to those of *Lathyrus* poisoning. On the other hand Acton and Chopra maintain that an amine is responsible for the toxicity of *Lathyrus sativus,* while other investigators could find no traces of poisonous bases in the various *Lathyrus spp.* examined by them.

Anderson and his co-workers could find no alkaloids in the unripe and ripe seeds of *Lathyrus sativus* and consider that the alkaloids isolated by Stockman and Dilling were contained in extraneous seeds contaminating the samples of *Lathyrus sativus* used in their investagations.

Stockman (1929) mentions that in a publication of 1917 he isolated a poisonous alkaloid from the seeds of *Lathyrus sativus* and that Dilling isolated two poisonous alkaloids from the seeds of this plant. In his 1929 publication Stockman states that the active principle of *Lathyrus* is soluble in cold water, acidified water, weak and 90 per cent. alcohol, and chloroform. In his 1931 publication Stockman withdraws his statement made in 1917 that the active principle of *Lathyrus sativus* is of an alkaloidal nature and now states that, according to his investigations, the toxic constituent of *Lathyrus sativus* grown in India and *L. cicera* grown in France is an acid. He has succeeded in isolating a poisonous acid also from *Ervum ervilia* L. (bitter vetch), *Ervum lens* (lentils), *Pisum sativum* (common pea), *Soya hispida* (soy bean), *Vicia sativa* (tares) and *Cajanus indicus* (pigeon pea). He states that the poisonous acid in these beans is probably the same.

B. SYMPTONS.

Lathyrus is derived from the Greek word " Lathyros " meaning vetchling. " Lathyros " is stated to have its root in a Greek word meaning exciting and impetuous. Greek and Roman agricultural history refers to the stimulant properties of pulses for man and domestic animals.

Human beings: Poisoning is of most frequent occurrence in young men ; women, children and elderly men are less susceptible. The increased susceptibility of the former is supposed to be due to exposure to cold and wet weather and fatigue.

It rarely happens that prodromal symptoms of pain, numbness, cramps and prickling are experienced, the most common course of the disease being a sudden onset of weakness and heaviness in the legs and loins. Walking is impaired, reflexes are increased, the muscles tremble when weight is put on them. If the consumption of *Lathyrus* is not discontinued, paralysis will progress until the patient is unable to walk. The arms are rarely affected in the same way as the legs. Convulsive movements of the upper and lower limbs and painful contractions of the muscles are experienced.

Post-mortem appearances : Pronounced atrophy of the spinal cord. Histological examination reveals disappearance of cells in the affected portion of the nervous system and increase in neuroglia (the picture resembling that of a recovered case of myelitis transversa) (Petri, 1930).

The affected muscles are atrophied and show fatty degeneration.

Domestic animals: The symptoms in domestic animals to a very large extent resemble those exhibited by human beings, weakness and paralysis of the hindquarters being the most common symptom. In horses which are the most susceptible of our domestic animals. " roaring " is frequent, which is caused by paralysis of the nervus recurrens and acceleration of the pulse, due to incipient paralysis of the vagus centre. Prodromal symptoms of excitement may occur. Death usually follows after months of illness with symptoms of asphyxia.

Bovines exhibit symptoms similar to those seen in horses except "roaring." They show suspended rumination, constipation, paralysis of the limbs, small and weak pulse and loss of sensibility in the skin. Monkeys are affected in the same way as human beings.

Sheep, pigs, dogs, ducks, geese, peacocks and pigeons all develop weakness and paralysis with their accompanying symptoms.

C. POST-MORTEM APPEARANCES AND HISTOLOGY.

There is atrophy of the larynx muscles and degenerative changes in the ganglion cells of the spinal cord and vagal and accessory nuclei of the medulla. Thickening of the walls of the arterioles and capillaries in the spinal cord and degeneration of the myocard have been described.

In the horse the following lesions were found: congested patches in the stomach and intestines, hyperaemia of the lungs and catarrhal bronchitis: and in cattle: thick and dark blood, a large amount of bloody serum in the cranium and anterior portions of the spinal canal, pronounced congestion of the meninges with haemorrhagic patches.

D. TREATMENT.

Feeding of the *Lathyrus spp.* must immediately be discontinued. Medicinal treatment may consist of applying stimulants to the central nervous system (strychnine) and irritants (mustard plasters, etc.) to the skin along the spinal column.

ONDERSTEPOORT EXPERIMENTS.

As *Lathyrus sativus* appeared to be less susceptible to pests attacking other winter crops, there was a possibility of its being extensively grown in South Africa as a winter feed for stock. It was for this reason that it was decided to ascertain the toxicity (if such existed) of this plant in all its stages of development, both in the fresh and dried state. Unfortunately, owing to the enforcement of stringent economic measures it was possible only to conduct feeding experiments with the plant in the fresh state and preflowering and flowering stages.

Horses, cattle, sheep and rabbits were used in these experiments. These animals were offered daily the freshly cut plant without any additional ration and every twenty-four hours after feeding the remaining quantities of plant material were weighed so as to calculate the amount of plant eaten in twenty-four hours. The amounts of plant recorded in the table given below are approximate as in feeding experiments there unavoidably is a certain error in the difference of the weights of the fresh succulent plant fed and the remaining quantity weighed twenty four hours after feeding owing to loss of moisture and wastage during feeding. It is obvious that the more succulent the plant is and the slower the ingestion, the greater this error will be.

The material fed consisted of a mixture of the plant in the preflowering and flowering stages.

The results of the above experiments are recorded in the following table :—

TABLE I.

LATHYRUS SATIVUS FEEDING EXPERIMENT.

Animal and D.O.B. No.	Age.	Weight at beginning of experiment.	Weight at end of experiment.	Average quantity of plant eaten daily.	Period of feeding.	Total quantity of plant consumed.	Result.
		Kg.	Kg.	Kg.	Days.	Kg.	
4 rabbits....	Fullgrown	2·1 2·8 2·4 2·3	2·0 2·7 2·5 2·4	1·145	89	102	On the third day of the experiment one rabbit was found lying with its head resting on the cage floor. It was unable to lift the head or sit up or move about and died at 4 p.m. the same day. *Post-mortem appearances* :— Slight hyperaemia of lungs. All other organs appeared normal. It was substituted by another fullgrown rabbit. The remaining rabbits developed no symptoms.
2 rabbits....	Fullgrown	2·8 2·6	2·9 2·9	1·183	71	84	Remained healthy.
2 rabbits....	Fullgrown	2·7 2·6	2·8 2·5	1·270	63	80	Remained healthy.
Sheep 29652..	Fullmouth	33·0	34·0	4·76	55	262	Remained healthy.
Sheep 23555..	Fullmouth	45·0	46·0				
Horse 18237..	Aged.....	--	---	3·26	35	114	Died on 38th day of the experiment.
Horse 18526..	Aged.....	—	—	3·85	55	212	Developed symptoms of poisoning but recovered.
Ox 3636.....	± 2 years	273	298	26·86	22	591	Remained healthy.
Heifer 4199..	± 2 years	245	248				

All the animals, with the exception of the horses which did not take the plant readily the first few days of the experiment, ingested the plant with eagerness throughout the period of experimentation.

RABBITS.

Of a group of four rabbits which ingested the plant at the rate of 1·145 Kg. daily, one animal showed a sudden onset of paralysis on the third day of the experiment and died the same day. The post-mortem revealed nothing but a slight hyperaemia of the lungs. Whether this rabbit possessed an idiosyncrasy for *Lathyrus* poison or died from some other cause, is difficult to say. The remaining eight rabbits remained in a perfect state of health in spite of the fact that they ingested large amounts of the plant over prolonged periods.

SHEEP.

Two sheep consumed 262 Kg. of the plant in fifty-five days without showing any ill-effects.

HORSES.

The average daily amount of plant ingested by the horses is small as compared with that ingested by the other animals owing to the fact that very little of it was taken from the time the horses developed symptoms of poisoning.

From the second week of the experiment both horses steadily lost in condition, and from the twenty-fifth day they showed an increase in the pulse rate and a fairly profuse diarrhoea. Five days later diarrhoea still persisted and both animals showed pronounced weakness of the hindquarters, dirty brown conjunctiva with a yellowish tinge, and laboured respiration. Horse 18237 appeared much worse than 18526, the former showing progressive paralysis. On the thirty-second day of the experiment it made repeated attempts to rise but without success. It was repeatedly seen sitting up like a dog. Its condition was poor, respiration laboured, pulse weak and accelerated and the conjunctiva dirty yellowish brown and showed ecchymoses. The animal yawned frequently and at times made chewing movements. In the course of another two days the animal was completely paralysed, the prominent parts of the body showing abrasions due to struggling. Death occurred on the thirty-fifth day of the experiment after the animal had ingested 114 Kg. of the plant.

Post-mortem appearances: Abrasions on all prominent parts of the carcase; intense general icterus; hyperaemia of the lungs; subepicardial haemorrhages; pigmentation and degenerative changes in the liver; blood not coagulated and tarry in consistence; gastrophilus larvae in stomach; impaction of caecum, which contained a large amount of grit; chronic catarrhal enteritis.

Histology: Dr. G. de Kock, Head of the Department of Pathology, Onderstepoort, who examined the specimens collected from horse 18237, reported that no specific changes were seen in the organs. The liver and kidneys showed hyperaemia.

Horse 18526, which received 212 Kg. in the course of fifty-five days, developed the same train of symptoms. It showed pronounced weakness of the hindquarters and was frequently seen supporting its balance by leaning against the stable wall. As no more plant material was available, the feeding had to be discontinued with the result that improvement set in, the animal appearing quite normal a month after discontinuation of the feeding.

CATTLE.

Two young bovines ingested 591 Kg. of *Lathyrus sativus* in twenty-two days without having suffered any ill-effects.

In the following table are recorded the results* of a chemical examination of *Lathyrus sativus* :—

TABLE II.

Nature of plant material.	Origin of plant material.	Ash.	P_2O_5.	CaO.	Fat.	Cellulose.	Protein.	Moisture content.
Fresh green plant in flowering stage	Onderstepoort	% 6·0	% 0·51	% 1·08	% 4·47	% 36·6	% 17·3	% 74·5 air dried.
Hay (flowering and seeding plant)	Cotton Breeding Station, Barberton	8·0	0·34	0·96	2·4	27·0	20·9	8·3
Seed (ripe).............	Cotton Breeding Station, Barberton	2·9	0·74	0·24	0·6	—	28·0	10·0

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* I am indebted to Mr. D. J. R. van Wyk of the Division of Chemistry, Department of Agriculture, Pretoria, for these analyses.

With regard to the protein and fat content of *Lathyrus sativus* it has a feeding value equal to, if not better than other vetches.

DISCUSSION.

The first question that arises is whether *Lathyrus sativus* is poisonous or not. As botanical identifications are by no means perfect, it is highly probable that the various investigators in the different parts of the world have been working with different species or varieties of *Lathyrus*, which were all identified as *Lathyrus sativus*. As consignments of seeds were forwarded to some investigators the probability of these consignments containing seeds other than those of *Lathyrus sativus* is even greater, unless such seeds were collected by a competent person from properly weeded lands. It is therefore clear that it is of the utmost importance to preserve specimens of all plants used in experiments in order to be able to compare such plants with those about whose identity doubt may arise at a future date.

There is also little doubt that the contradictory results recorded in the literature were due, at least partly, to the various consignments of seeds being obtained from the plant grown in different parts of the same country or even different countries as it is a well established fact that the same plant grown in different localities may vary in toxicity to a considerable extent.

The plant grown at Onderstepoort from seed obtained from the Cotton-Breeding Station, Barberton, was identified as *Lathyrus sativus* by the Division of Botany, Pretoria, and specimens of this plant are being kept in the National Herbarium, Pretoria (No. 11766) and at the Onderstepoort Herbarium (O.P. Spec. No. 1388; 5/6/31). It must be mentioned that at Onderstepoort the *Lathyrus sativus* seeds were planted in rows eighteen inches apart which allowed of proper weeding and at no time was the material fed contaminated with any extraneous plants.

Onderstepoort experiments have proved beyond doubt that this plant grown at Onderstepoort in the period May–December, 1931, and in the state and stages in which it was used in the experiments, was posionous to horses, causing death in one and serious symptoms of poisoning in another. The symptoms exhibited by these two animals were identical with those described in the literature in *Lathyrus sativus* poisoning, with the exception of the general icterus found in the Onderstepoort cases. Also no "roaring" was present in the Onderstepoort cases.

Another point which coincided with information supplied in the literature is that horses are the animals most susceptible to *Lathyrus sativus* poisoning. Rabbits, sheep and bovines have consumed relatively much larger amounts of the plant grown at Onderstepoort than horses and have suffered no ill-effects. Not only did the plant cause no damage to the health of these animals but it apparently supplied all the food requirements essential for growth and maintenance of health.

The fact that *Lathyrus sativus* is poisonous when fed in large amounts and without any additional ration, does not preclude its use as a stock feed and as a part of the daily human diet. Experiments have proved, and this has been corroborated by numerous observations made in India and other countries, that *Lathyrus sativus* seeds can form part of the daily diet of man and animal with very beneficial results, provided moderate amounts are taken.

With regard to the nature of the toxic principle of *Lathyrus sativus* confusion exists as in the case with information concerning whether it is toxic or not. The same reasons, that have been given for the contradictory information in connexion with the toxicity of the plant, may be advanced here.

Some investigators have failed to isolate any toxic principle from this plant, whilst others state it to be prussic acid, a substance of alkaloidal nature, an amine and an acid respectively.

SUMMARY.

Lathyrus sativus grown at Onderstepoort and fed without any additional ration in the fresh state and in the preflowering and flowering stages proved to be poisonous to horses. Cattle, sheep and rabbits, although having consumed relatively larger amounts of the plant, suffered no ill-effects.

No definite results with regard to the active principle of *Lathyrus sativus* have been achieved by the various investigators.

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Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 1, Number 1, 1933.

Recent Investigations into the Toxicity of Known and Unknown Poisonous Plants in the Union of South Africa.

By D. G. STEYN, B.Sc., Dr.Med.Vet., Veterinary Research Officer, Onderstepoort.

(*Continued from the* 18*th Report D.V.S.* 1931.)

AIZOACEAE.

Psilocaulon absimile N.E. Br.

See the article "*Psilocaulon absimile* N.E.Br. as a Stock Poison" by C. Rimington and D. G. Steyn published elsewhere in this report.

ASCLEPIADACEAE.

Cynanchum capense Thunb.

Registered Number.—Onderstepoort Spec. No. 2406; 31/8/31. Nat. Herb. No. 14360.

Common Names.—Klimop.

Origin.—Baviaansdrift, P.O. Eastpoort, Bedford.

State and Stage of Development.—Wilted and in preflowering stage.

Sheep 29669.—Received 2,700 grams of wilted and dry leaves and stems in the course of five days.

Result.—Negative.

COMPOSITAE.

Senecio albanensis Harv. (Vix. DC.).

Registered Number.—Onderstepoort Spec. No. 5670; 4/2/32.

Common Names.— —

Origin.—"The Willows," P.O. Silverton, Pretoria.

State and Stage of Development.—Fresh and in flowering stage.

Sheep 28203.—Received 750 grams of the fresh plant on two consecutive days.

Result.—Negative.

Senecio venosus Harv.

Registered Number.—Onderstepoort Spec. No. 5669; 4/2/32.

Common Names.—

Origin.—"The Willows," P.O. Silverton, Pretoria.

State and State of Development.—Fresh and in late seeding stage.

Sheep 31877.—Received 1,500 grams of the fresh leaves, stems and seed-heads on two consecutive days.

Result.—Negative.

CRASSULACEAE.

Adromischus umbraticolis, C.A. Sm.

Registered Number.—Onderstepoort Spec. No. 6794; 31/3/32, Iron Peg No. 67.

National Herbarium Number.—12432.

Common Names.— —

Origin.—Magaliesberg, Pretoria North.

State and Stage of Development.—Fresh and in postflowering stage.

A 96 per cent. alcoholic extract of the leaves was prepared and injected subcutaneously into guinea pigs. 100 gram equivalent of fresh leaves caused attacks of clonic spasms closely resembling those seen in Cotyledonosis in guinea pigs, death occurring five hours after injection. 30 grams equivalent of fresh leaves had no effect on guinea pigs.

EXTRACT FROM THE MANUSCRIPT OF C. A. SMITH, AT THE NATIONAL HERBARIUM, PRETORIA.

Adromischus umbraticolus C.A. Sm.

The generic name *Adromischus*, was first assigned to several species of *Cotyledon* § *Spicatae* (sensu Fl. Cap. 11.370) by Lemaire (Jard. Fleur. II Misc. 59: 1852) but was not taken up either by Harvey in the "Flora Capensis" or by Schonland in his classic papers on the South African species of Cotyledon and it was not until recently that Berger resuscitated Lemaire's generic name (Engl. & Prantl. Nat. Pflanzenfam. VIII, a 416: 1930) under which he affected a number of new combinations for species previously described by other authors—Baker, Schonland, Marloth—under Cotyledon (§ Spicatae) as well as giving a key to the 15 known species.

The species represented by *Smith* 3432 and other specimens quoted below cannot be identified with any of these existing species and is therefore here described for the first time. In the National Herbarium it has previously been mistakenly identified as *Cotyledon trigyna* Burch.

A. umbraticolus sp. nov., affinis *A. trigono* (Burch) C. A. Sm., sed forma alternorum immaculorum foliorum differt.

Planta perennis, succulenta, parva. *Caulis* ad apicem foliosus, usque 12 cms. altus et 2 cm. crassus, simplex vel ramosus, teres. glabor. *Floria* alterna, carnosissima, ad apicem caulis (vel ramorum brevius) congregata, oblongocuneata usque obovata et ad basin cuneata, apice plerumque obtusa, usque 6 cm. longa, 2 cm. lata et (prope basin) 4 mm. crass, basi teretes, ad apicem planiores, superne convexa vel plana, infra convexa, immaculata sed saturate virides et saepe circum apicem purpurea vel rosea, omnio cereo pulvere tenuiter tecta. *Inflorescentia* terminalis, simplex ool 2–3– ramosa, laxe in dimidio anteriore racemosa, omnino ceres pulvere tenuiter tecta. *Flores* numerosi, singuli, pedicellati. *Pedicelli* ascendentes patentesque, usque 6 mm. (in fructu usque 1·5 cm.) longi, plerumque 2–3 bracteis; bracteis lanceolato-ovatae, usque 1·5 mm. longae, carnosae. *Dentes calycis* ovato-deltoidei, acuminati, usque 2·5 mm. longi, corolae adpressi. *Tubus corollae* cylindricus, rectus, obtuse 5–angulatus et 5 su.catus, usque 1 cm. longus, purpureus, fauce saturate purpureus, ovato-deltoideis usquo 2 mm. longis stellatis deinde reflexis delicatis purpureis lobis. *Filamenta* filiformia, purpurea; 4 plus minusve in medio tubo corollae exserta; 4 prope basin inserta, inclusa. *Ovaria* 4–5, oblongo-ovoidea, usque 5 mm. longa, in stylo subulato viride instructa. *Squamae nectarii* oblongae, usque 1·5 mm. longae, minute emarginatae, pallide virides.

Transvaal Highveld.—Pretoria Div.: On the Magaliesberg at Silikaats Nek, in rocky crevices on northern slopes, C. 1500 M., Nov. 1926, *Smith* 3432: (Type). In Wonderboompoort, along rocky ledges and in crevices of precipitous sides of cliffs, C. 1470 M., Dec. 1925, *Smith* 1766: At Pretoria, along north slope of Meintjes Kop range, in rocky crevices near the old fort, C. 1470 M., Sept. 1925, *Smith* 693A; in rocky fissures and crevices in rich humus, below the Reservoir, C. 1470 M., Nov. 1926, *Smith* 3456: and in eod. loc., Jun 1931, *Smith & Ward* 3: Witwatersrand Div.: Braamfontein, Johannesburg, on rocky hills, 1800 M., Dec. 1898, *Gilfillan* 60: Grown at Onderstepoort, Steyn in National Herbarium No. 12432. (N.B.—The specimens quoted are preserved in the National Herbarium, Pretoria.)

The above species is very common on the ranges round Pretoria and on the Magaliesberg, being nearly always found on rocky ledges with the rootsystems under rocks, or in the crevices of these rocks. The vegetative parts are almost invariably found in the shade of other plants, while the long peduncles thrust the opening flowers beyond the shade so as to render them accessible to insect visitors.

In the Meintjes Kop localities above cited the numbers quoted were found growing under moderate-sized specimens of *Combretum holosericeum* Sond., *C. zeyheri* Sond., *Burkea africana* Hook., *Strychnos pungens* Sol., *Vangeuria tomentosa* Hochst., and tangled masses of *Landolphia capensis* Oliv., being associated with specimens of *Crassula orgyrophylla* Diels (very generally), *Euphorbia schinzii* Pax (frequently), *Salacia regmanni* Schinz, *Kalanchoe paniculata* Harv., *Aloe davyana* Schönl., *Cyphocarpa augustifolia* Lopr., *Eulophia Hians* Spreng., *Pachystigma zeyheri* Sond., *Parinarium capense* Harv., *Leonotis microphylla* Skan., and *Cotyledon leucophylla*, C.A.Sm., along with several grasses.

In Wonderboompoort, the plants grow in large colonies and in one place, have completely overrun a fair area.

The leaves of the species behave like those of other species in the genus, e.g. *A. marianae* (marl.) Berger, for when they drop off fine „ rootlets " develop at the base, and eventually new leaves (in time giving rise to a fresh plant) appear.

CRUCIFERAE.

Lepidium draba Linn.

Registered Number.—Onderstepoort Spec. No. 3578; 24/10/31.

Common Names.— —

Origin.—" Carolspoort," De Aar.

State and Stage of Development.—Wilted and in flowering stage.

Rabbit.—Received 40 grams of the wilted leaves and flowers on each of two consecutive days.

Result.—Negative.

Sheep.—Received 8,700 grams of the wilted and dry leaves, stems and flowers in the course of seventeen days.

Result.—Negative.

CUCURBITACEAE.

Momordica foetida Schum. et Thonn.

Registered Number.—Onderstepoort Spec. No. 6795; 31/3/32.

Common Name.— —

Origin.—Pretoria North.

Portion of Plant Tested.—Fresh green fruit.

Rabbit.—Received 100 grams of the fresh green fruit on two consecutive days.

Result.—Negative.

DICHAPETALACEAE.

Dichapetalum cymosum (Hook.) Engl.

Registered Number.—Onderstepoort Spec. No. 5596; 27/1/32.

Common Names.—Gifblaar, blaargif, blinkblaar, magou, makou, " Umbetti " (native name in South West Africa).

Origin.—Grootfontein, South West Africa.

State and Stage of Development.—Dry mature leaves of plant in the fruiting stage.

This plant was suspected of having caused death in camels.

Rabbit.—Received 30 grams of dry leaves on one day.

Result.—Negative.

EQUISETACEAE.

Equisetum ramossissimum Desf.

Registered Number.—Onderstepoort Spec. No. M. 18/2/32.

Common Names.—Horsetail; perde stert; mare's tail; drilgras, dronkgras.

Origin.—Onderstepoort Poisonous Plant Garden (original specimens from C. J. G. Loock, "Lissie," P.O. Slabberts Siding, O.F.S.).

State and Stage of Development.—Fresh and forming fruit heads.

Sheep 31599.—Received 800 grams of the fresh plant at 12 noon, 18/2/33.

Result.—19/2/32.—Lying down repeatedly and rising again; laboured respiration; accelerated pulse; staggers when driven; slight fever; uncertain gait which resembles that of an animal with sore feet. Received another 800 grams of fresh plant.

20/2/32.—Whole body trembles when standing; symptoms show a slight amelioration; feet do not appear "sore" any more; lying down most of the time.

21/2/32.—Improving.

22/2/32.—Appears normal.

GENTIANACEAE.

Chironia Transvaalensis Gilg.

Registered Number.—Onderstepoort Spec. No. 5671; 4/2/32.

Common Names.— —

Origin.—"The Willows," P.O. Silverton, Pretoria.

State and Stage of Development.—Fresh and in flowering stage.

Rabbit.—Received 10 grams of the wilted leaves and flowers on each of three consecutive days.

Result.—Negative.

Rabbit.—Received 25 grams of the wilted leaves and flowers on 24/2/32.

Result.—25/2/32.—Appears very ill, extremely laboured respiration; accelerated and weak heartbeat; general weakness (paresis); staggers about; unable to keep head up; died at 10.30 a.m.

Post Mortem Appearances.—General cyanosis; heart in systole; hyperaemia of lungs and liver; acute catarrhal gastritis with numerous haemorrhages in the mucosa; slight acute catarrhal duodenitis.

Rabbit.—Received 30 grams of the fresh leaves in one dose.

Result.—Negative.

Rabbit.—Received 100 grams of the fresh leaves, stems and flowers on one day.

Result.—6/2/32.—Found dead at 7 a.m.

Post Mortem Appearances.—General cyanosis; marked hyperaemia and slight oedema of the lungs; dilatation of heart ventricles; small and big intestine distended with a large amount of very fluid contents.

Sheep 24273.—Received 400 grams of the slightly wilted leaves, stems and flowers at 12 noon, 22/2/32.

Result.—4 p.m.—Apathetic; laboured respiration; accelerated pulse.

23/2/32.—Lying down; apathetic; laboured respiration: cyanosis; frothing at the mouth; tympanitis; weak and accelerated pulse; died at 4.30 p.m.

Post Mortem Appearances.—Intense general cyanosis; congestion of subcutaneous bloodvessels especially those of the front quarters; dilatation of heart ventricles; degenerative changes in myocard; pronounced hyperaemia and slight oedema of the lungs; oedema of perportal lymphglands; hyperaemia of and haemorrhages in retropharyngeal and submaxillary lymph glands; degenerative changes in liver; hyperaemia of abomasal mucosa; slight acute catarrhal duodenitis and jejunitis.

Sheep 21409.—Received 800 grams of the fresh leaves, stems and flowers at 12 noon 20/2/32.

Result.—9 p.m.—Animal appears in distress; laboured respiration; accelerated pulse; cyanosis.
21/2/32.—Found dead at 6.30 a.m.

Post Mortem Appearances.—Resembled that of sheep 24273 very closely.

Scilla sp. (near *Scilla Cooperi* Hook.).

Registered Number.—Onderstepoort spec. No. 5660; 4/2/32. Nat. Herb. No. 13397.

Common Names.— —

Origin.—Piet Retief.

State and Stage of Development.—Leaves wilted, bulbs fresh in postflowering stage.

Rabbit.—50 grams of bulbs and wilted leaves in one dose.

Result.—Negative.

Rabbit.—100 grams of bulbs and wilted leaves on one day.

Result.—Died the following night.

Post Mortem Appearances.—General cyanosis; slight oedema and pronounced hyperaemia of lungs; marked dilatation of heart ventricles.

Sheep 32313.—Received 800 grams of wilted leaves and bulbs at 12.45 p.m. 4/2/32.

Result.—4 *p.m.*—Laboured respiration; accelerated and strong pulse; foaming at the mouth; apathetic; inappetence.
5/2/32.—Above symptoms and frequent urination.
6/2/32.—Above symptoms and frequent urination.
7/2/32.—Condition improving.
8/2/32.—400 grams of wilted leaves and bulbs at 2 p.m. 3.30 p.m. same symptoms as at 4 p.m. on 4/2/32.
13/2/32.—Animal appears normal.

Ornithogalum caudatum Ait.

Registered Number.—Onderstepoort Spec. No. 12388; 29/12/31. Nat. Herb. No. 12388.

Common Names.— —

Origin.—Entembeni, Hluhluwe, Zululand.

State and Stage of Development.—Fresh and in preflowering stage.

Rabbit.—Received 40 grams of the fresh leaves and bulbs on each of two consecutive days.

Result.—Negative.

Ornithogalum lacteum Jacq.

Registered Number.—Onderstepoort Spec. No. 0; 29/9/31. Nat. Herb. No. 10056.

Common Names.—Chinkerinchee.

Origin.—Onderstepoort Poisonous Plant Garden (bulbs originally from "Skilpadbeen," Willowmore).

State and Stage of Development.—Fresh and in flowering stage.

Rabbit.—Received 40 grams of fresh leaves, flowers and bulbs.

Result.—Negative.

Ornithoglossum glaucum Salisb.

Registered Number.—Onderstepoort Spec. No. 625; 13/5/32. Nat. Herb. No. 11547.

Common Name.—Cape Slangkop.

Origin.—Prieska.

State and Stage of Development.—Fresh and in postseeding stage.

Rabbit.—Received 30 grams of fresh leaves in one dose.

Result.—Pronounced laboured respiration two hours after dosing; weak and accelerated heartbeat; apathetic; died four hours after dosing.

Post Mortem Appearances.—Pronounced hydroperitoneum; hyperaemia of gastric mucosa; advanced cirrhosis of the liver.

The advanced cirrhosis of the liver most probably rendered this rabbit more susceptible to this plant.

Rabbit.—Received 50 grams of fresh leaves in one dose, at 12 noon, 13/5/32.

Result.—14/5/32.—Apathetic; dyspnoea; weak and accelerated heart beat; profuse diarrhoea.
15/5/32.—Died at 7.30 a.m.

Post Mortem Appearances.—Cyanosis; hyperaemia and slight oedema of lungs; dilatation of heart ventricles; subserosal haemorrhages (stomach); acute catarrhal gastro-enteritis; degenerative changes in the liver.

Scilla sp.—(Probably undescribed).

Registered Number.—Onderstepoort Spec. No. 5250; 2/12/31. Nat. Herb. No. 12389.

Common Names.— —

Origin.—" Vaalbank," Wakkerstroom.

State and Stage of Development.—Fresh and in flowering stage.

Rabbit.—Received 30 grams of fresh bulbs and leaves.

Result.—Negative.

OLEACEAE.

Ligustrum lucidum Ait.

Registered Number.—Onderstepoort Spec. No. 1058; 28/5/32. Nat. Herb. No. 11565.

Origin.—" Sandfontein," Daspoort, Pretoria.

Common Name.— —

Rabbit.—Received 75 grams of the fresh ripe fruit in one dose.

Result.—Negative.

PORTULACACEAE.

Talinum caffrum E. and Z.

Registered Number.—Onderstepoort Spec. No. 6172 (*b*); 25/2/32.

Common Name.— —

Origin.—Palla Ranch, P.O. Debeeti, Tuli Block, Bechuanaland Protectorate.

State and Stage of Development.—Dry and in early fruiting stage.

Rabbit.—Received 30 grams of the fresh rootstock.

Result.—Negative.

Rabbit.—Received 40 grams of dry leaves and stems.

Result.—Negative.

RUBIACEAE.

Pygmaeothamnus chamaeodendron (O. Kuntze) Robyns.

Registered Number.—Onderstepoort Spec. No. 5572; 2/2/31. Nat. Herb. No. 11057.

Common Name.— —

Origin.—" Waveney," Newcastle, Natal.

State and Stage of Development.—Dry and in flowering and early fruiting stage.

Sheep 31785.—Received 3,600 grams of the dry leaves and flowers in the course of twelve days.

Sheep 31465.—Received 7,200 grams of the dry leaves and flowers in the course of twelve days.

Result.—Both sheep showed intermittent fever for about two months after the dosing had been discontinued. Sheep 31465 developed diarrhoea, which lasted two days.

SOLANACEAE.

Nicandra physaloides Gaertn.

Registered Number.—Onderstepoort Spec. No. P.; 10/3/32.

Common Name.—Apple of Peru.

Origin.—Onderstepoort Poisonous Plant Garden. (Seed obtained from plants sent in from Ixopo, Natal.)

State and Stage of Development.—Fresh and in fruiting stage.

Sheep 31599.—Received 1,200 grams of the fresh leaves, stems and green fruit in two doses administered on one day.

Result.—Negative.

Rabbit.—Received 100 grams of the fresh leaves on one day.

Result.—Negative.

Nicotiana glauca R. Grah.

Registered Number.—Onderstepoort Spec. No. N.; 18/1/32.

Common Names.—Wilde tabak, wild tobacco.

Origin.—Onderstepoort Poisonous Plant Garden.

Rabbitt.—Received 5 grams of ripe seeds.

Result.—Negative.

Rabbit.—Received 20 grams of ripe seeds on each of two consecutive days.

Result.—Negative.

Physalis minima Linn.

Registered Number.—Onderstepoort Spec. No. 1149; 2/6/32.

Common Names.—Wilde gooseberry, wilde appelliefie, kalkoengif.

Origin.—" Zoals-hy-lykt," Amersfoort.

Rabbit.—Received 150 grams of fresh ripe berries in one dose.

Result.—Negative.

Solanum auriculatum Ait.

Registered Number.—Onderstepoort Spec. No. R.; 2/12/32. Nat. Herb. No. 11404.

Common Name.— —

Origin.—Onderstepoort Poisonous Plant Garden (seeds obtained from plants growing at Warner Beach, Natal).

State and Stage of Development.—Plants 12-18 inches high and in preflowering stage.

Rabbit.—Received 55 grams of fresh leaves on one day.

Result.—Negative.

Solanum supinum Dunal.

Registered Number.—Onderstepoort Spec. No. 5341 ; 8/1/32.

Common Names.—Bitter appel ; bitter apple.

Origin.—"Rocklands," P.O. Klipdam, Griqualand West.

Rabbit.—Received 30 grams of the fresh ripe fruit on each of two consecutive days.

Result.—Negative.

Withania somnifera Dunal.

Registered Number.—Onderstepoort Spec. No. S. ; 12/2/32.

Common Name.—Geneesblaren.

Origin.—Onderstepoort Poisonous Plant Garden.

Rabbit.—Received 120 grams of fresh green berries on one day.

Result.—Negative.

THYMELAEACEAE.

Gnidia capitata Linn.

Registered Number.—Onderstepoort Spec. No. T. ; 1/10/31.

Common Name.— —

Origin.—Onderstepoort.

State and Stage of Development.—Fresh and in flowering stage.

Rabbit.—Received 60 grams of fresh leaves, stems and flowers, in one dose.

Result.—Negative.

VITACEAE.

Cissus hereroensis Schinz.

Registered Number.—Onderstepoort Spec. No. 5317 ; 8/1/32.

Common Name.—Lakseer bossie.

Origin.—"Rocklands," P.O. Klipdam, Griqualand West.

State and Stage of Development.—Fresh and in postflowering stage.

Rabbit.—Received 60 grams of fresh leaves in the course of two days.

Result.—Negative.

SUMMARY.

The toxicity of twenty-seven plant specimens was investigated and of these the following were found poisonous :—

(*a*) ***Psilocaulon absimile*** N.E. Br.
(*b*) ***Adromishcus umbraticolus*** C.A. Sm. (slightly toxic).
(*c*) ***Equisetum ramossissimum*** Desf.
(*d*) ***Chironia transvaalensis*** Gilg.
(*e*) ***Scilla sp.*** (Nat. Herb. No. 13397).
(*f*) ***Ornithoglossum glaucum*** Salisb.

Equisetum ramossissimum Desf. administered in the fresh state produced symptoms in sheep closely resembling those attributed to this plant.

No previous records of toxicity of the following plants could be found in the available literature :—

Psilocaulon absimile N.E. Br.
Chironia Transvaalensis Gilg.

LITERATURE.

Same as that quoted in previous reports.

ACKNOWLEDGMENTS.

I am indebted to Dr. E. P. Phillips, Principal Botanist, Division of Plant Industry, Pretoria, for the identification of Plant specimens.

Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 1, Number 1, 1933.

Fungi in Relation to Health in Man and Animal.

By D. G. STEYN, B.Sc., Dr.Med.Vet., Veterinary Research Officer, Onderstepoort.

I.—INTRODUCTION.

A.—GENERAL.

The fungi harmful to man and animal may be divided into three main groups according to the nature of their effects:—

(*a*) Those growing on the skin and mucous membranes and in the organs, for example, those causing ringworm and aspergillosis.

(*b*) Those, which grow "wild" and contain toxic substances within themselves, for example, poisonous mushrooms (toadstools).

(*c*) Poisonous symbiotic and parasitic fungi attacking foodstuffs:

  (1) Those containing the poison within themselves (ergot).

  (2) Those producing harmful substances in the foodstuffs on which they grow.

The fungi concerned in groups (*a*) and (*b*) have been the subject of many investigations and are extensively referred to in the literature. The information obtained through these investigations is definite and conclusive, whilst our knowledge of the fungi concerned in group (*c*), and the results obtained by experiments with these fungi, are inconclusive, contradictory and by no means definite. It is for these reasons that it is proposed to deal only with the last group of fungi.

The following literature may be consulted for information with regard to the first two groups of fungi:—

***Group** (a).*—**Pammel (1911), Fröhner (1919), Fröhner (1922), Seiffert (1920), Byam and Archibald (1921), Hutyra and Marek (1922), Romanov (1928), Bull (1929), Schäfer (1929), Whitfield (1929), Woolridge (1929), Morris (1931), Steyn (1931), Datta (1932), Seren (1932), and Thomson and Fabian (1932).**

Seren (1932) gives an excellent historical review of fungi causing disease through growing in animal and human tissues; also his experiments are of great interest.

***Group** (b).*—**Bruinsma (1906), Kobert (1906), Pammel (1911), Heffter (1924, Vol. 2, No. 2), Silberbauer and Mirvish (1927), Petri (1930), and Glaister (1931).**

B.—HISTORICAL.

Group A (c).—Fungus-infected Foodstuffs.

Husemann, Hilger and Husemann (1882) refer to cases of poisoning in Italy caused by bread prepared from fungus-infected maize, and state that an extract prepared from this bread was poisonous and contained an alkaloidal substance.

These authors refer to a bitter, loliin, which is contained in the seed of *Lolium temulentum* Linn. (darnel, drabok), but make no mention of fungi being concerned in the causation of the toxicity of darnel.

Kobert (1906) states that in poisonous darnel there is a fungus between the hyaline layer and the aleuron cells and that this fungus does not affect the viability of the plant. Antze claimed to have isolated two alkaloids, loliin and temulentin, and temulentic acid from darnel. Hofmeister found a crystalline base, temulin ($C_7H_{12}N_2O$), which was absent in fungus-free darnel, to be the active principle. Temulin has narcotic and mydriatic effects, and the lethal dose for cats is 0·25 gm. per Kg. bodyweight. According to Hofmeister fungus-infected darnel does not contain more than 0·06 per cent. of the alkaloid temulin. In France it is held that darnel is most poisonous before it ripens.

Quoting Woronin, Kobert continues that in Russia fungus-infected rye was responsible for darnel-like poisoning in human beings. The rye grains were infected with *Fusareum roseum* Link, "*Gibberella saubinitii*" (ascophoric form of the former), "*Helminthosporum herbarum*," "*Epicoccum neglectum*," "*Trichothecium roseum*," "*Eurotium herbariorum*" (ascophoric form of "*Aspergillus glaucus*"), "*Hymenula glumarum*," "*Cladochytrium graminis*", etc.

Similar symptoms in human beings and in animals were caused by rye in France in 1890. All these rye grains were infected with the same fungus, which Prillieux and Delacroix termed "*Endoconidium temulentum*" (in the conidia form).

Kobert also refers to the toxic nature of the so-called sleepy grass ("*Stipa vaseyi*"), and suggests that its toxicity is due to a parasitic fungus. Marsh and Clawson (1929) have, however, proved this plant toxic to horses and sheep and make no mention of the presence of fungi on the plant.

Pammel (1911) refers at length to fungi. Certain fungi (for example, "*Mucor Rouxii*" in China) are used in the preparation of yeast and alcoholic drinks as they change starch into sugar, which in turn liberates alcohol on further fermentation. In experiments conducted by Gamgee in 1868 two cows were fed a mixture of corn meal, hay and corn smut (*Ustilago zeae* Beck). The ration of the one animal was moistened whilst the other received the mixture in a dry state. In the course of three weeks these two animals consumed forty-two pounds of corn smut. The cow receiving the dry ration lost in condition despite a voracious appetite, whilst the other animal remained in good health and gained in weight. Further cases are quoted by Pammel in which corn smut was fed to heifers without any ill-effects. Corn smut fed in large amounts also to cows for a period of forty-nine days produced no harmful effects. The milk yield was normal and none of the experimental animals aborted. In addition to these experiments numbers of others, in which cattle have ingested large amounts of corn smut, are referred to.

Ustilago avenae (Pers.) Jens. (oat smut) is said to produce a sore throat when present in large quantities. The sore throat is caused by irritation and is not due to this fungus growing in the mucous membrane. Barley smut [*Ustilago nuda* (Jes) Kell and Sw.], wheat smut [*Ustilago tritici* (Pers.) Jens.] and *Ustilago panici-glauci* (Wallr.) resemble oat smut in its harmful effects on man and animal. From the last-named fungus Power isolated a small amount of ergotin and some farmers consider that this fungus causes abortion.

Tilletia foetens (B and C) Trel.(stinking smut or bunt) and *Tilletia Tritici* (Bjerk) Wint (wheat bunt) are referred to as causing a bad colour and dark colour in flour, but nothing is mentioned about their toxic properties.

The rust of oats, wheat and grass (*Puccinia spp.*) are liable to cause severe irritation of the buccal and nasal mucous membranes.

Referring to *Penicillium glaucum* Link. (blue mould) Pammel (1911) says that it is widely distributed on decaying fruit and certainly is not pathogenic apart from the fact that it may sometimes cause mycotic stomatitis. *Aspergillus glaucus* (L.) Link is stated not to be pathogenic but probably causes the development of a poisonous substance in the substratum. " Staggers " or " mad staggers " (enzootic cerebritis), is said to be caused by feeding maize, which is attacked by this fungus. Reference is also made to the possibility of its causing mycotic stomatitis.

Pammel (1911) and Long (1917) refer to *Claviceps purpurea* (Fr.) Tul. Pammel states that *Diplodia zeae* (Schw.) Lév. occurs in the ears of mealies and may be responsible for forage poisoning. Foodstuffs attacked by *Fusarium roseum* Link are supposed to be poisonous. Pammel also refers to the toxicity of darnel being due to a fungus.

Brown (Editorial. 1916) found a " yeast " (Torula) and two moulds, namely "*Mucor erectus*" and "*Aspergillus oryzae*" in the root of *Mesembryanthemum Mahoni* N.E. Br., from which the natives of South Africa prepare a fermenting agent, which is used in the manufacture of a drink named " Khadi " and as a rising principle for bread.

Fröhner (1919) states that "*Claviceps purpurea*" (ergot) usually occurs on rye but may also be found on wheat, oats, barley and grasses. As active principles he describes conutin (or secacornutin), which causes contraction of the uterus: sphazelinic acid, which produces necrosis due to hyaline degeneration and thrombosis of the peripheral arterioles: and ergotic acid, which is a narcotic and has no action on the uterus. The symptoms of ergot poisoning are described as follows: gastro-intestinal irritation; necrotic stomatitis; gangrene and mummification of extreme parts of the body: uterus contractions; and nervous disturbances. This fungus, which in itself is poisonous, has been responsible for many cases of poisoning in man and animal. Fröhner describes botanic and spectroscopic methods for the detection of ergot in grain and hay.

Fröhner (1919) attributes the following symptoms to foodstuffs attacked by "*Mucor mucedo,*" "*Mucor racemosus,*" "*Mucor stolonifer,*" "*Mucor phycomyces,*" "*Aspergillus glaucus,*" "*Penicillium glaucum*" and "*Oidium lactis*": inappetence, colic, tympanites, constipation and diarrhoea. In some cases salivation, difficult deglutition, vomiting, icterus, polyuria, nephritis, cystitis, dizziness, staggering, pronounced depression, paralysis of the extremities, hindquarters, tongue, urinary bladder, ears and retina, and general paralysis were present. Pushing, bellowing, shivering, convulsions, epilepti-form spasms, profuse perspiration, imperceptible pulse, reddish-brown discolouration of the conjunctiva and rapid loss in condition were also seen.

The post-mortem lesions described are : gastro-enteritis with haemorrhages and erosions : accumulation of serum in the brain cavities and in the arachnoidal sac ; hyperaemia and oedema of the brain and spinal cord ; haemorrhagic fluid in the peritoneal cavity ; cystitis, nephritis ; peritonitis and acute yellow atrophy of the liver. In some cases the autopsy is negative.

" *Tilletia Caries* " (on wheat), " *Ustilago carbo* " (on wheat, oats, barley and pasture grasses), " *Ustilago moidis* " (on mealies), " *Ustilago longissima* " (on grasses) and " *Ustilago echinata* " (on *Phalaris* and *Phragmites spp.*) are held responsible for the following symptoms in animals (Fröhner, 1919): paralysis of pharynx, oesophagus and tongue ; weakness ; swaying gait ; staggering : complete paralysis of the motor and sensory nerves ; constipation ; diarrhoea, swelling of the eyelids : lachrymation ; and catarrh of the upper air-passages ; and pregnant animals may abort.

The post-mortem in many cases reveals no lesions. Sometimes irritation of the gastro-intestinal tract and the air passages are found.

The following symptoms are ascribed by Fröhner (1919) to foodstuffs infected with *Puccinia graminis, Puccinia straminis, Puccinia coronata, Puccinia arundinacea,* and *Uromyces apiculatus :* inflammation of the skin, lips, cheeks, eyelids, and head ; urticaria ; severe itching ; conjunctivitis and inflammation of the buccal mucous membrane, pharynx and gastro-intestinal tract.

The post-mortem reveals, apart from the skin lesions, haemorrhagic gastro-enteritis, nephritis and cystitis, and haemorrhages in the serous membranes.

Furthermore, Fröhner mentions that the following fungi are poisonous : "*Polydesmus exitiosus*" (on rape), "*Polythrincium trifolii*" (on clover) and "*Epichloë typhina,*" (on grasses). The symptoms produced by foodstuffs attacked by these fungi are similar to those described for *Ustilago* and *Puccinia spp.*

The treatment consists in the administration of purgatives, calomel being preferred on account of its disinfecting properties ; furthermore, creatin, and, as chemical antidotes, tannin, tannoform and iodine. For the rest the treatment is symptomatic.

Fröhner also refers to the fact that foodstuffs infected with these fungi can at times be taken in large amounts with impunity.

Fröhner considers the suggestions that darnel is toxic only when infected with a fungus as acceptable.

Furthermore, Fröhner (1919, p. 391) states that (a) "*Poa aquatica*" attacked by " *Ustilago longissima,*" and (b) " *Phragmites communis* " infected with "*Puccinia arundinacea*" are poisonous. "*Peronospora viticola* " causes colic, tympanites, constipation, diarrhoea and abortion in cows. " *Peronospora Herniariae* " and " *Peronospora Viciae* " are also stated to be poisonous.

Mitchell [1918 (a)] produced hyperaesthesia, inco-ordination of movements, muscular tremors, dyspnoea and an accelerated and weak pulse in cattle by feeding them on the fungus " *Claviceps paspali,*" which is of frequent occurrence on " *Paspalum dilatatum* " pastures. He was unable to produce abortion in pregnant animals and mummification as are found in " *Claviceps purpurea* " poisoning. A meal of nine pounds of *paspalum* heads, which were badly attacked by the fungus, sufficed to produce well-defined symptoms, whilst 8 oz. of pure sclerotia caused quite recognizable symptoms. The symptoms appeared two days after feeding and recovery was very slow.

Mitchell (1918) proved that maize infected with "*Diplodia zeae*" causes inco-ordination of movement and paralysis in cattle. He was able to produce symptoms of poisoning within two days after having fed 20 ℔. of artificially infected maize to bovines.

Mitchell (1918) also fed 19½ ℔. of maize artificially infected with "*Mucor mucedo*" to an ox without the animal suffering any ill-effects.

Fröhner (1922) adds the group of yeast fungi (*Hefepilze*) to the four groups described by him in his text book on toxicology (Fröhner, 1919). These yeast fungi are responsible for alcohol fermentation. He ascribes the following symptoms of poisoning in animals to foodstuffs, in which this group of fungi have caused fermentation: cerebral stimulation with subsequent narcosis and paralysis.

Hutyra and Marek (1922) (Vol. 3, p. 187) state that according to observations "*Ustilago Maidis*" causes gout in birds.

Thomson and Sifton (1922) discuss poisoning by ergot and fungus-infected foodstuffs and refer to the fact that foodstuffs infected with certain moulds have been found poisonous whilst on other occasions they proved innocuous when fed in large mounts, although the same fungi were present. A specific case is mentioned in which a strain of "*Aspergillus fumigatus*" from Italy was very poisonous whilst the same strain obtained from Germany was very slightly or not at all toxic.

The experiments of Graham, Bruechner and Pontius (Thomson and Sifton, 1922) with mouldy hay have thrown much light on some mysterious cases of poisoning with fungus-infected foodstuffs. They isolated an anaerobic botulinus-like organism from mouldy hay, which had caused typical cases of forage poisoning. The moulds present in the hay apparently provide the necessary anaerobic conditions for the growth and propagation of this botulinus-like bacillus. Graham and his collaborators furthermore produced typical forage poisoning by feeding pure cultures of this bacillus to animals and also succeeded in producing immunisation to this organism. The symptoms of poisoning described closely resemble those described by Theiler and his collaborators (1927) in parabotulism (Lamsiekte) on South Africa.

Danckwortt (1926) sounds a warning note with regard to the use of solvents in the extraction of oil from foodstuffs, for example, the use of ether and trichlorethylene. Ether is easily decomposed by air and light and poisonous substances may be the result as has been proved to be the case. Trichlorethylene may exert poisonous effects as, owing to its enormous surface area on the foodstuffs, it is absorbed very rapidly. Poisoning with such extracted foodstuffs may erroneously be attributed to fungi which may be present on these foodstuffs.

Jarmai (1925) produced visceral and articular gout in geese by force-feeding them with maize infected with "*Penicillium glaucum*." Negative results were, however, obtained when an artificial culture of this fungus was fed to or injected intravenously into fowls. Furthermore, cases of uraemia in ducks caused by mouldy maize and in pigeons caused by mouldy wheat are mentioned.

Lander (1926) refers to the toxicity of ergot and darnel, but makes no mention of the toxicity of the latter being due to a fungus.

Heyne (1927) mentions that *Mucor dubius* Wehmer, *Mucor javanicus* Wehmer and *Rhizopus oryzae* Wert et Prinsen Geerlings are used in China and Java in the preparation of yeast *ragi*. The last-named fungus renders the less digestible ingredients of legume seeds more assimilable.

Theiler (1927) describes deplodiosis (*Diplodia zeae* Schwz. Lev) in cattle and sheep. Feeding experiments with pigs and horses were negative. Three pounds of mealies infected with "*Diplodia zeae*" ingested in three days caused poisoning in sheep. Two pounds of maize artificially infected with this fungus caused poisoning in sheep within two days. The symptoms disappear within a few days after the discontinuation of the feeding with "*Diplodia zeae*" infected maize.

No symptoms of poisoning could be produced by feeding the mycelium alone.

The symptoms described by Theiler closely resemble those reported by Mitchell (1918). The post-mortem revealed catarrhal enteritis and hyperaemia of the lungs and kidneys.

Elsässer (1928) describes vomiting and a foetic diarrhoea in pigs and, in those with an unpigmented skin, an intensely itching vesicular eczema after feeding on mouldy barley that had been imported from America.

Lührs (1928) also refers to this fungus-infected American barley, which caused vomiting in pigs. Lührs attempted feeding this barley to pigs but they refused to take it. A pig was then drenched with 1 pound of this barley and vomiting occurred within fifteen minutes after drenching.

Lührs considered biogenic amines, for example, cholin, responsible for the toxic effects of this barley, and described a method of differentiating between normal barley and the mouldy American barley. He was able to detoxicate this barley by boiling it with a 2 per cent. sodium carbonate and then neutralising with 10 per cent. hydrochloric acid and again boiling for thirty minutes.

Stang [1928 and 1928 (a)] states that horses, cattle, sheep and laboratory animals took the mouldy American barley which caused vomiting in pigs, with impunity.

Stang [1928 (a)] examined the barley microscopically and found hyphae and spores of "*Fusarium roseum*" whose form of development occurred as "*Gibberella Saubinettii.*"

Danckwortt (1929) found up to 0·095 per cent. ammonia in the harmful American barley whilst normal barley contained 0·073 per cent. He, therefore, confirmed Altenberg's findings that there is no appreciable increase in the ammonia or volatile amine bases in mouldy rye. Danckwortt also found no difference of any significance in the sulphuretted hydrogen content of the American barley and normal barley.

The following is quoted from an abstract (Editorial 1929) dealing with the abovementioned mouldy American barley: "On the other hand, the investigation of similar samples by the Director of the German Institute of Milling failed to reveal any fungus or toxamine, but showed the presence of undetermined bacteria which fermented barley dough with a copious production of gas and caused a repulsive butyric acid-like smell. It is believed that these bacteria cause fermentation and the decomposition of proteids in the animal stomach, the products of which are toxic to pigs, animals which are known to be highly susceptible to this kind of poisoning."

Miessner and Schoop (1929) detected spores of "*Fusarium roseum*" in the mouldy American barley to which reference has already been made and succeeded in growing this fungus on artificial media. The following fungi were found in specimens of this barley: a red pigment forming yeast, "*Cladosporium herbarum,*" "*Alternaria spp.,*" and "*Fusarium roseum.*"

These fungi proved to be non-pathogenic to guinea pigs and mice. The three first named fungi had no detrimental effect on pigs to whose ration large amounts of pure cultures of these fungi were added; also when parenterally administered they produced no ill-effects. "*Fusarium roseum*" cultures added to the foods of pigs caused these animals to refuse the food altogether. Pigs dosed by means of a stomach tube with large amounts of "*Fusarium roseum*" cultures showed increased defaecation and inappetence for twenty-four hours.

Miessner and Schoop regarded "*Fusarium roseum*" as the causative factor in the production of poisonous substances in this mouldy American barley.

Oppermann and Doenecke (1929) also refer to this harmful American barley.

The following is a passage from a publication by Dickson and his co-publishers (1930): "The water extract from barley severely infected by scab ("*Gibberella Saubinettii*") finely ground and extracted for four to six hours, was found to produce acute vomiting in pigs, and became more active when freed from protein, polysaccharides and nitrogenous substances precipitable with tannic acid. The active substances or materials appear to be associated with the fractions containing glucoside or basic nitrogen compounds."

Heller, Caskey and Penquite (1930) during their investigations into the toxicity of smuts have suffered ill-effects due to the inhalation of the smut spores. These ill-effects were sensitivity of the upper pulmonary tract, increased heart action. headache and feeling of nausea. These authors investigated the effects of grain-sorghum smuts on rats, guinea pigs rabbits. fowls. horses and cows. Rats, guinea pigs, rabbits and fowls were fed on a well-balanced diet to which these smut spores had been added. In addition sorghum plants of which fully 70 per cent. had smut-infected heads, were fed to horses, milk cows and young cattle. All the above animals were fed over prolonged periods without any ill-effects as to their condition, growth, milk yield, egg yield and reproduction.

Müller (1930) attributed the following symptoms in a horse to poisoning with hay infected with *Aspergillus* (definite species not mentioned): colic, inappetance, pushing against stable wall, pronounced perspiration, muscular tremors, extreme excitement, pronounced salivation, dyspnoea, fever, cyanosis, frequent urination. charging with head against stable wall, turning somersaults, staggering, falling, exhaustion, and an imperceptible pulse. Death occurred twenty-four hours after the onset of symptoms.

Autopsy revealed gastro-enteritis; meningoencephalitis acute haemorrhagica; incipient acute swelling of the spleen; and turbid swelling of the liver, kidneys and myocard. Müller found the hay, part of which this animal had consumed, infected with "*Aspergillus*" and considered this the cause of death.

Mundkur and Cochran (1930) write: "During the autumn of 1928, hogs and poultry in Iowa were extensively poisoned by the consumption of barley contaminated by *Gibberella Saubinettii* the perithecia of which were present on the surface of 4·8 per cent. of the grain. Feeding experiments were carried out on hogs, chickens and guinea pigs, all of which rejected an exclusive barley diet, while the two first-named developed symptoms of nausea and lost weight."

Roche, Rokstedt and Dickson (1930) state that cattle, sheep and poultry on Wisconsin farms may be fed on barley infected with "*Gibberella Saubinettii*" (scab) without suffering any ill-effects. whereas pigs, dogs, horses and man are susceptible to low percentages of badly scabbed kernels. They suggest that scab infected fodder be fed to ruminants and poultry and in this way prevent wastage.

Barger (1931) published an excellent monograph in which he discusses the historical, botanical, chemical, pharmacological and clinical, and the pharmaceutical and forensic aspects, of "Ergot and Ergotism." See article "Poisoning of Human Beings by Weeds contained in Cereals (Bread poisoning)" published elsewhere in this Report (Darnel poisoning).

Bürgi (1931) states that "*Mucor*," "*Penicillium*" and "*Aspergillus*" are to be considered with regard to poisoning caused by fungus-infected foodstuffs and also refers to the fact that animals may at times ingest such foodstuffs over prolonged periods without any apparent detrimental effects.

Bürgi has seen several cases of colic in horses as a result of eating fungoid hay and also gastro-enteritis and other symptoms of poisoning, most with exitus letalis, in horses which had taken mouldy bread.

Bürgi, furthermore, states that with regard to fermented hay the following organisms (according to Düggeli) are concerned: "*Mucor*," "*Aspergillus*," "*Bacterium coli*," "*Oidium lactis*," *Bacterium fluorescens*," "*Bacillus mesentericus*" and "*Actinomyces thermophyles liquefaciens*." Most of these organisms are destroyed at a temperature of 75°C. The oxidation process, however, continues on pure chemical lines through the catalytic action of iron and manganese combinations, with the result that the temperature in fermenting hay stacks may reach 280°C. From this moist mass water, carbon dioxide, saltpetre combinations, karomel, furfurol, acetic and formic acid can be distilled. Furfurol on aldehyde in cheap alcoholic drinks has been proved poisonous to dogs and fowls.

Bürgi also refers to poisoning with fresh hay, the symptoms of which are inappetence, drowsiness, fever, accelerated pulse, colic, diarrhoea, tympanites, heart weakness and abdominal pulsation. Death occurs with symptoms of dyspnoea and heart failure. On autopsy pronounced hyperaemia of the small intestine and oedema of the lungs were present.

It was thought that cumarin is the cause of the trouble but Fröhner found that cumarin and other scents of fresh hay cause more or less severe intestinal irritation, but no actual poisoning. Poisoning with fresh hay is, therefore, not clearly understood.

Mains, Vestal and Curtin (1931) fed "*Gibberella Saubinettii*" infected barley to pigs with the result that these animals took scarcely enough to maintain life. Amounts of this barley, not exceeding 10 per cent. of the total ration, were however, safely given to pigs. This barley, which contained 58 per cent. of scab, was successfully fed to cattle as 50 per cent. of the grain ration, and to fowls as 20 per cent. of the ration.

Richner (1931) states that mustiness and mouldiness in cereal seed grain are liable to occur when the moisture content of the grain exceeds 15 per cent. The principle fungi concerned were "*Penicillium*," "*Citromyces*," and "*Aspergillus*," followed by "*Mucor*" spp., "*Rhizopus nigricans*," "*Fusarium culmorum*," "*Fusarium herbarum*," "*Alternaria tenuis*," "*Dematium pullulans*," "*Trichothecium roseum*" and "*Actinomycetes*."

Shofield (Roderick and Schalk, 1931) produced a very acute anaemia in rabbits with a destruction of 50 per cent. of the red blood cells by feeding these animals on aqueous extracts of mouldy sweet clover. The clotting time of the blood was delayed. Unfortunately no reference is made to the fungi concerned.

Csukas (1932) describes cases of acute inflammation of the upper air-passages in horses and in some of the human beings attending these horses. In each case he found the hay or straw infected with fungi, but he was unable to produce this affection experimentally with the suspected fungus infected hay or straw. No mention is made of the kinds of fungi or organisms concerned, except in one case in which streptococci, staphylococci, actinomyces, streptrotrix and a thick rod-like bacterium were isolated from the bedding straw.

Riedl (1932) describes laminitis in horses due to the feeding of fermented sugar beet.

(C.) THE TOXIC CONSTITUENTS OF FUNGI AND FUNGUS-INFECTED FOODSTUFFS.

The toxic constituents of fungi and fungus-infected foodstuffs can according to the information at our disposal, be divided into (*a*) those that are produced and contained in the fungus itself (*Claviceps purpurea* and *Claviceps paspali*) and (*b*) those that are produced by fungi growing on foodstuffs and causing certain chemical changes in the constituents of such foodstuffs.

(a) *Toxic constituents of* Claviceps purpurea *and* Claviceps paspali.

Barber (1931) and all text-books on pharmacology and toxicology deal extensively with ergot (*Claviceps purpurea*) and ergotoxine, ergotinine, tyramin and histamin are generally accepted as its active constituents (Milks, 1930). Mitchell (1918), who proved *Claviceps paspali* toxic to cattle, makes no reference to its active principles.

Heffter (1923) mentions that Dale and Ewins detected acetylcholin in ergot. Acetylcholin is much more poinonous than cholin, but is very unstable. The symptoms of poisoning are similar to those causes by cholin. Recently it was found that the action of ergot as an ecbolic is due in the first place to an "unknown constituent" and not to ergotoxine and ergotamin (Editorial, 1932).

Gieger (1932) separated an amorphous alkaloid from the oil of *Claviceps paspali*. Less than 0·1 gm. of this alkaloid proved fatal to a guinea pig.

(b) *The toxic constituents of fungus-infected foodstuffs.*

This aspect of mouldy foodstuffs has been repeatedly investigated with varying and inconclusive results.

Bodin and Gautier (Pammel, 1911, p. 265) state that "*Aspergillus fumigatus*" produces a bacteria-like toxin when grown on media containing a mixture of protein and carbohydrate. This toxin exerts its chief actions on the nervous system causing muscular convulsions. Rabbits and dogs are much more susceptible to this toxin than guinea pigs, cats, mice and white rats.

Fröhner (1919) states that poisoning with fungi is caused not by their spores entering the blood circulation in a physical way but by toxins formed by the fungi. The toxin of smuts has an irritant action on mucous membranes and a paralytic effect on the centre of deglutition and the spinal cord. Very little is known of the production and nature of fungus toxins, probably these are products of decomposition of constituents of the substances on which the fungi grow. Leber found irritant toxins, resembling the phlogosin of streptococci and staphylococci, in cultures of "*Aspergillus fumigatus*" and "*Penicillium glaucum*," whilst Buss detected a poisonous substance in "*Oidium albicans*" (Fröhner, 1919).

Seiffert (1920) mentions that Ceni and Besta, and Bodin extracted specific toxins from the mycelia of "*Aspergillus fumigatus*."

Czapek (1921) states that the formation of oxalic acid by fungi growing on sugar containing media is a well-known phenomenon. "*Peziza sclerotiorum*" (*Sclerotinia Libertiana* Fuck), forms more oxalic acid from sugar when the substratum contains calcium than when it is calcium-free. It is stated that "*Aspergillus niger*" forms oxalic acid not only when growing on substrata containing sugar but also on those containing salts of organic acids, albumoses, amino acids, and to a lesser extent on those containing glycerine and vegetable fats. The origin of the nitrogen is of importance in the formation of oxalic acid by fungi on sugar substrata in as much as no oxalic acid is formed when ammonium chloride or ammonium sulphate is added, whilst it is readily liberated when peptone serves as origin of the nitrogen. It is furthermore mentioned that "*Saccharomyces Hansenii*" forms oxalic acid from sugars and carbohydrates.

Many investigators consider that the toxicity of fungus-infected foodstuffs is due to the fungi secreting toxins on the feed (Thomson and Sifton, 1922).

Danckwortt (1926) accepts that fungi themselves are non-poisonous and that they cause the formation of harmful substances in the substrata. These substances probably are products of decomposition of proteins contained in the foodstuffs attacked by fungi. Biogenic amines, some of which are poisonous, may be formed. Abderhalden proved amino acids harmless to dogs.

Of the biogenic amines, which are likely to be formed in fungus-infected foodstuffs and which may be responsible for the poisoning caused by such foodstuffs, cholin, which is a product of decomposition of phosphatides, has received most attention.

Cholin is a constituent of most plant cells and is also present in many animal tissues to the extent of 0·01 to 0·03 per cent. Cholin is not considered very poisonous as it is rapidly oxidised in the animal tissues. Frogs are killed by 0·1 to 0·25 gm. cholin chloride administered subcutaneously. There is cessation of respiration and death with paralysis. 0·5 to 1·0 gm. per Kg. body-weight administered to mammals causes salivation, lachrymation, increased peristalsis, weakness of extremities and death with symptons of respiratory paralysis. When administered intravenously spasms occur before death. Cats are more susceptible than rabbits (Heffter, 1923).

Sollman (1922, p. 325) gives the lethal intravenous dose of Cholin for mammals as 1-2 mg. per Kg. body-weight.

Cholin is a parasympathetic stimulant, like muscarin, with many of its actions similar to those of pilocarpine and physostigmine.

According to Trier (1931) putrefactive organisms decompose cholin into the ten times more poisonous neurin.[*]

Neither Brieger nor Ruckert nor Vogt succeeded in isolating neurin from cultures of "*Oidium lactis,*" "*Cholrera virrions,*" "*Penicillium*" or "*Algae,*" which were allowed to decompose through bacterial action (Heffter, 1923).

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[*] Führer (1932) found 0·09–0·1 mg. acetylcholin per gram bodyweight lethal to mice and 0·2–0·23 mg. per gram bodyweight lethal to frogs (*Rana temporaria*).

Schroeter and Strassburger (1931) extracted from mouldy American barley a poisonous substance, which on subcutaneous administration to frogs and mice caused spasms and paralysis, which in most cases ended fatally within ten to eighteen hours. Control extracts from healthy barley proved to be non-toxic.

Schroeter and Strassburger found free cholin in the above barley. They fed cholin-butyric-acid-ester to pigs with negative results. On injecting subcutaneously into 30-35 Kg. pigs 0·4 gm. cholin-butyric-acid-ester chlorhydrate in 10 c.c. distilled water the following symptoms were developed: immediate restlessness, squealing, climbing the walls of the sty, extremely accelerated pulse within one minute after injection, pronounced dyspnoea and collapse. Improvement set in within thirty minutes after injection, complete recovery resulting within eight hours.

From the results of their investigations Schroeter and Strassburger concluded that cholin or some easily hydrolysable cholin-butyric-acid-esters were the cause of the harmfulness of the mouldy American barley.

Starek [Schroeter and Strassburger (1931)] suggests that the fungi or bacteria found on the American barley decompose lecithin into cholin or easily hydrolysable esters of cholin.

According to Boehm (Schroeter and Strassburger, 1931) cotton-seed cakes contain toxic amounts of free cholin.

Theiler (1927) reported negative results with regard to feeding experiments on sheep with the mycelium of "*Diplodia zeae.*" Van der Byl found aldehyde, alcohol and acids in the cultures of this fungus. Theiler suggests that under certain conditions large amounts of alcohol and aldehyde may be formed by this fungus and may then prove harmful. The intestinal catarrh caused by "*Diplodia zeae*" infected mealies was, according to Theiler, due probably to the acids present in the cultures.

Lührs (1928) refers to the fact that healthy barley contains 0·035 per cent. cholin and states that, according to his investigations, there was a marked increase in the cholin content of the harmful mouldy American barley due to the action of the fungi. He continues that owing to the fact that pigs are very susceptible to these biogenic amines, it is quite acceptable that cholin is the toxic ingredient of this barley as only pigs were affected.

Danckwortt (1929) examined the already referred to mouldy American barley chemically. He excluded poisoning with heavy metals, alkaloids and prussic acid. He found no appreciable difference in the ammonia and hydrogen sulphide content of this barley and normal barley. Unfortunately it is at present still impossible to determine by chemical means qualitatively and quantitatively the labile biogenic amines.

According to Wernery (1929) trimethylamin was detected in a specimen of bran which had caused harmful effects in cows and which contained many micro-organisms. Altenburg Wernery, (1929) isolated tyramin from the substrata of fungi cultures.

Trimethylamin, which is a typical product of decomposition of all proteids, causes increased reflexes, accelerated respiration and pronounced convulsions, which are followed by deep coma.

Tyramin, which is a very common product of protein putrefaction, has an adrenaline-like action and also causes contraction of the uterus.

Dickson and his co-publishers (1930) found the active substances of the above harmful mouldy American barley to be associated with those fractions of the extracts containing glucoside or basic nitrogen compounds.

According to Bürgi (1931) Valentin detected in fungi cultures alkaloidal substances, which had an irritant and lethal action on experimental animals. Bürgi also mentions that Di Pietro isolated from the spores of *Penicillium toxicum* a glucoside, which caused spastic-paretic symptoms with an increase in reflexes and muscular tone. Ceni and Besti (Bürgi, 1931) isolated from "*Aspergillus fumigatus*" and "*Aspergillus flavescens*" a spasm-producing toxin and a paralytic toxin from "*Aspergillus niger*" and "*Penicillium*" spp.

With regard to poisoning with fresh hay it was thought that cumarin, the characteristic sweet smelling constituent of fresh hay, was responsible for the harmful effects, but Fröhner found cumarin and other scents of fresh hay not to have any detrimental effects on the system apart from causing a more or less severe intestinal irritation.

Roderick and Schalk (1931) administered cumarin per os in large amounts over prolonged periods to rabbits with no apparent ill-effects. They were unable to extract the toxic principle of damaged sweet clover.

Seren (1932) quotes the views of Valentin, Leber, di Pietro, Ceni and Berta, and Bodin and Gautier, with regard to the toxic constituents of mouldy foodstuffs. These views have already been referred to.

D.—IMMUNITY TO POISONING WITH FUNGUS-INFECTED FOODSTUFFS.

(a) *Natural Immunity.*

In plant poisoning acquired immunity and acquired tolerance must be clearly distinguished from each other. The term acquired immunity should be used only in connection with plants which contain toxalbumins as active principles, which cause the development of antibodies in the body, whilst acquired tolerance should be spoken of in connection with those plants, which when repeatedly taken by animals, cause the development of a resistance other than that brought about by the production of antibodies. As the nature of the active principles produced in foodstuffs by the growth of fungi is unknown only the term immunity will be used in this article.

Mitchell (1918) writes: "Horses, mules and donkeys would appear not to be susceptible, as these animals graze over the same area where cattle are dying from the disease without suffering any harmful results. No cases have been recorded of goats or pigs having been affected."

Thomson and Sifton (1922) state that horses and mules are the animals chiefly affected, cattle, sheep, pigs and poultry being apparently very resistant, if not immune to the effects of fungi.

Theiler (1927) recorded negative results in feeding experiments with "*Diplodia zeae*" infected maize on horses and pigs, whilst cattle and sheep developed well-marked symptoms and even died.

Stang (1928) states that horses, cattle, sheep and laboratory animals took the mouldy American barley in all forms, whereas pigs refused it and when forced to take it the latter animals developed inappetence and some even vomited.

Roche, Boksteds and Dickson (1930) referring to scab infected barley write: "It has been found that cattle, sheep, and poultry on Wisconsin farm may be fed on barley infected with scab (*Gibberella Saubinettii*) without adverse effects, whereas pigs, horses and dogs, as well as man, cannot tolerate even low percentages of badly scabbed kernels. It is suggested that a considerable saving may be effected by the utilization of infected fodder for the ruminants and poultry, since it must otherwise be sold at a heavy discount."

Mains, Nestal and Curtis (1931) state that barley containing up to 58 per cent. of scab ("*Gibberella Saubinettii*") was successfully fed to cattle as 50 per cent. of the grain feed ration and to fowls as 20 per cent. of the ration, whilst pigs hardly took enough of this barley to maintain life.

(b) *Acquired Immunity.*

Mitchell (1918) expressed the view that an ox which developed no symptoms of poisoning although it had ingested a larger quantity of Diplodia-infected maize than the two bovines which developed symptoms, may have developed a tolerance resulting from an attack in the preceding year.

Seiffert (1920) failed to produce an immunity in guinea pigs to "*Aspergillus fumigatus*" by injecting them subcutaneously and intravenously with virulent material and also subcutaneously with nucleoproteid solutions prepared from "*Aspergillus fumigatus*" and subsequently testing their immunity by injecting virulent spore emulsions intravenously.

II.—ONDERSTEPOORT EXPERIMENTS.

Specimens of fungus-infected bran, mealies, hay and ensilage, which were suspected of having caused harmful effects in human beings (maize) and stock (bran, mealies, hay and ensilage), have repeatedly been received at Onderstepoort for investigation.

Maize showing a 100 per cent. infection of fungi was fed to fowls (60 grams) and to rabbits in quantities up to 847 gm. without any deleterious effects. No additional food was given during the period of feeding.

In the following table is contained a summary of the experiments conducted by the author in the course of the last four years at Onderstepoort with fungus-infected foodstuffs.

EXPERIMENTS WITH FUNGUS-INFECTED FOODSTUFFS CONDUCTED AT ONDERSTEPOORT.

Animal.	Method of administration.	Material fed or drenched.	Period of feeding or drenching.	Total amount fed or drenched.	Result.
Rabbit A.....	Fed (no additional food given)	100 + mouldy bran infected with *Rhizopus* (probably *R. nigricans*) a *Fusarium* sp. and bacteria	16 days.....	1070 grams..	Negative.
Rabbit B.....	„ „	*Fusarium moniliforme* culture on maize. (No. 550—see Addendum by Dr. Doidge.)	7 days.....	680 grams..	„
Rabbit C.....	„ „	„ „ „	7 days.....	1000 grams..	„
Rabbit D.....	Per stomach tube	*Fusarium moniliforme* culture on Raulin's medium. The " mould skin " was separated from the fluid, washed several times in tap water and given to this animal (No. 550)	3 days.....	60 grams..	„
Rabbit E.....	„ „	„ „ „	„	„	„
Rabbit F.....	„ „	The fluid, from which the " mould skin " drenched to rabbits D and E had been removed, was given to this rabbit	3 days.....	240 c.c.....	„
Rabbit G.....	„ „	„ „ „	„	„	„
Rabbit H....	„ „	Pure Raulin's medium (control)................	2 days.....	240 c.c.....	„
Rabbit I.....	„ „	„ „	„	„	„
Rabbit J.....	Fed (no additional food given)	*Fusarium moniliforme* var. *sub-glutinans* culture on maize (No. 602)	7 days.....	900 grams..	„
Rabbit K.....	Per stomach tube	*Fusarium moniliforme* var. *sub-glutinans* culture on Raulin's medium. The mould skin was separated from the fluid, washed several times in tapwater and then given to this animal	3 days.....	60 grams..	On the fourth day of the experiment this animal was found partially paralysed; death occurred the same day. *Post-mortem appearances :* Pronounced hyperaemia of lungs, dilation of both heart ventricles.

EXPERIMENTS WITH FUNGUS-INFECTED FOODSTUFFS CONDUCTED AT ONDERSTEPOORT—(*continued*).

Animal.	Method of administration.	Material fed or drenched.	Period of feeding or drenching.	Total amount fed or drenched.	Result.
Rabbit L.....	Per stomach tube	*Fusarium moniliforme* var *sub-glutinans* culture on Raulin's medium. The mould skin was separated from the fluid, washed several times in tapwater and then given to this animal	3 days.....	60 grams..	On the eleventh day of the experiment this animal was partially paralysed, the paralysis progressed until animal was prostrate and unable to move. It died on the third day after onset of symptoms. *Post-mortem appearances.* Pronounced hyperaemia of lungs and liver, dilatation of both heart ventricles—urinary bladder markedly distended with ordinary urine.
Rabbit M.....	Per stomach tube	The fluid, from which the " mould skin " drenched to rabbits K and L had been removed, was given to this rabbit	3 days.....	240 c.c.....	Negative.
Rabbit N.....	" "	" " "	"	"	"
Rabbit O.....	Fed (no additional food given)	*Fusarium graminearum* Schabe culture on maize (No. 622)	7 days.....	600 grams..	"
Rabbit P.....	" "	" " "	"	310 grams..	"
Rabbit Q.....	Per stomach tube	*Fusarium graminearum* Schwabe culture on Raulin's medium. The " mould skin " was separated from the fluid, washed several times in tap water and then given to this animal (No. 622)	3 days.....	60 grams..	"
Rabbit R.....	" "	" " "	"	"	"

EXPERIMENTS WITH FUNGUS-INFECTED FOODSTUFFS CONDUCTED AT ONDERSTEPOORT—(*continued*).

Animal.	Method of administration.	Material fed or drenched.	Period of feeding or drenching.	Total amount fed or drenched.	Result.
Rabbit S.....	Per stomach tube	The fluid, from which the "mould skin" drenched to rabbits Q and R had been removed, was drenched to this animal	3 days.....	240 c.c.....	Negative.
Rabbit T.....	„ „	„ „ „	„	„	„
Rabbit U.....	„ „	*Fusarium moniliforme* var. *subglutinans* culture on Raulin's medium. The "mould skin" was separated from the fluid, washed repeatedly in tap water and drenched to this rabbit (No. 631)	4 days.....	80 grams..	„
Rabbit V.....	„ „	„ „ „	„	„ ..	„
Rabbit W....	„ „	The fluid, from which the "mould skin" drenched to rabbits U and V had been removed, was given to this rabbit	5 days.....	300 c.c.....	„
Rabbit X.....	„ „	„ „ „	„	„	„
Rabbit Y.....	„ „	*Fusarium moniliforme* var. *subglutinans* cultures on Raulin's medium. The "mould skin" was separated from the fluid, washed repeatedly in tap water and drenched to this rabbit (No. 632)	5 days.....	100 grams..	„
Rabbit Z.....	„ „	„ „ „	„	„ ..	„
Rabbit Aa....	„ „	The fluid, from which the "mould skin" drenched to rabbits Y and Z had been removed, was given to this animal	5 days.....	300 c.c.....	„
Rabbit Ab....	„ „	„ „ „	„	„ ..	„
Rabbit Ac....	„ „	*Fusarium moniliforme* var. *subglutinans* culture on Raulin's medium. The "mould skin" was treated as above and given to this animal (No. 665)	5 days.....	100 grams..	„
Rabbit Ad....	„ „	„ „ „	„	„ ..	„
Rabbit Ae....	„ „	The fluid, from which the "mould skin" drenched to rabbits Ac and Ad had been removed, was given to this animal	5 days.....	300 c.c.....	„
Rabbit Af....	„ „	„ „ „	. .....	„	„

EXPERIMENTS WITH FUNGUS-INFECTED FOODSTUFFS CONDUCTED AT ONDERSTEPOORT—(*continued*).

Animal.	Method of administration.	Material fed or drenched.	Period of feeding or drenching.	Total amount fed or drenched.	Result.
Rabbit Ag....	Per stomach tube	*Fusarium moniliforme* var. *subglutinans* culture on Raulin's medium. The "mould skin," treated as above, was given to this animal (No. 284)	5 days.....	100 grams..	Negative.
Rabbit Ah....	,, ,,	,, ,, ,,	,, ...	,, ..	,,
Rabbit Ai....	,, ,,	The fluid, from which the "mould skin" drenched to rabbits Ag and Ah had been removed, was given to this rabbit	5 days.....	300 c.c.....	,,
Rabbit Aj....	,, ,,	,, ,, ,,	,, ...	,, ...	,,
Rabbit Ak....	,, ,,	*Fusarium graminearum* Schwabe culture on Raulin's medium. The "mould skin," treated as above, was given to this rabbit (No. 622)	2 days.....	120 grams..	,,
Rabbit Al....	,, ,,	The fluid, from which the "mould skin" drenched to rabbit Ak had been removed, was given to this rabbit	2 days.....	200 c.c.....	,,
Rabbit Am...	,, ,,	*Fusarium moniliforme* var. *subglutinans* culture on Raulin's medium. The "mould skin," treated as above was given to this rabbit (No. 222)	2 days.....	120 grams..	,,
Rabbit An....	,, ,,	The fluid, from which the "mould skin" drenched to rabbit Am had been removed, was given to this rabbit	2 days.....	200 c.c.....	,,
Rabbit Ao....	,, ,,	*Fusarium moniliforme* var. *subglutinans* Wr. and Rkg. culture on Raulin's medium. The "mould skin," treated as above, was given to this animal (No. 223)	,, ...	120 grams..	,,
Rabbit Ap....	,, ,,	The fluid, from which the "mould skin" drenched to rabbit Ao had been removed, was given to this rabbit	,, ...	200 c.c.....	,,
Rabbit Aq....	,, ,,	*Fusarium moniliforme* var. *subglutinans* Wr. and Rkg. culture on Raulin's medium. The "mould skin," treated as above, was given to this animal (No. 224)	,, ...	120 grams..	,,

EXPERIMENTS WITH FUNGUS-INFECTED FOODSTUFFS CONDUCTED AT ONDERSTEPOORT—(*continued*).

Animal.	Method of administration.	Material fed or drenched.	Period of feeding or drenching.	Total amount fed or drenched.	Result.
Rabbit Ar....	Per stomach tube	The fluid, from which the "mould skin" drenched to rabbit Aq had been removed, was given to this rabbit	2 days.....	200 c.c.....	Negative.
Rabbit As....	„ „	*Fusarium moniliforme* Sh. culture on Raulin's medium. The "mould skin," treated as above, was given to this rabbit (No. 220)	„	120 grams..	„
Rabbit At....	„ „	The fluid, from which the "mould skin" drenched to rabbit As had been removed, was given to this rabbit	„	200 c.c.....	„
Rabbit Au....	„ „	*Fusarium moniliforme* var. *subglutinans* culture on Raulin's medium. The "mould skin," treated as above, was given to this rabbit (No. 602)	1 day......	60 grams..	„
Rabbit Av....	„ „	The fluid, from which the "mould skin" drenched to rabbit Au had been removed, was given to this rabbit	1 day......	80 c.c.....	„
Rabbit Aw ..	„ „	8 day old *Fusarium moniliforme* var. *subglutinans* culture on mealie meal (No. 602)	2 days.....	100 grams..	„
Rabbit Ax....	„ „	16 day old *Fusarium moniliforme* var. *subglutinans* culture on Raulin's medium. The "mould skin," treated as above, was given to this rabbit (No. 602)	1 day.....	50 grams..	„
Rabbit Ay....	„ „	The fluid, from which the "mould skin" drenched to rabbit Ax had been removed, was given to this rabbit	1 day......	80 c.c.....	„
Rabbit Az....	Fed (no additional food given)	Mealie meal infected with *Fusarium moniliforme* var. *subglutinans* (No. 602)	13 days.....	1230 grams..	„
Rabbit Ba....	„ „	16 day old *Fusarium moniliforme* var. *subglutinans* culture on mealie meal (No. 602)	2 days.....	110 grams..	„
Rabbit Bb....	„ „	20 day old *Fusarium moniliforme* var. *subglutinans* culture on Raulin's medium. The "mould skin," treated as above, was given to this rabbit (No. 602)	1 day......	60 grams..	„
Rabbit Bc....	„ „	The fluid, from which the "mould skin" drenched to rabbit Bb had been removed was given to this rabbit	1 day......	120 c.c.....	„

EXPERIMENTS WITH FUNGUS-INFECTED FOODSTUFFS CONDUCTED AT ONDERSTEPOORT—*(continued)*.

Animal.	Method of administration.	Material fed or drenched.	Period of feeding or drenching.	Total amount fed or drenched.	Result.
Rabbit Bd....	Fed (no additional food given)	20 day old *Fusarium moniliforme* var. *subglutinans* culture on maize (No. 602)	2 days.....	100 grams..	Negative.
Rabbit Be....	„ „	25 day old *Fusarium moniliforme* var. *subglutinans* culture on Raulin's medium. The " mould skin," treated as above. was given to this rabbit (No. 602)	1 day......	60 grams..	„
Rabbit Bf....	„ „	The fluid, from which the " mould skin " drenched to rabbit Be had been removed, was given to this rabbit (No. 602)	„	120 c.c.....	„
Rabbit Bg....	„ „	25 day old *Fusarium moniliforme* var. *subglutinans* culture on maize, kept at temperature of 36°C. for last five days (No. 602)	2 days.....	100 grams..	„
Rabbit Bh....	„ „	25 day old *Fusarium moniliforme* var. *subglutinans* culture on Raulin's medium, kept at room temperature. The " mould skin," treated as above, was given to this rabbit (No. 602)	1 day......	60 grams..	„
Rabbit Bi....	„ „	The fluid, from which the " mould skin " drenched to rabbit Bh had been removed, was given to this rabbit (No. 602)	„	120 c.c.....	„
Rabbit Bj....	„ „	25 day old *Fusarium moniliforme* var. *subglutinans* culture on maize kept at room temperature (No. 602)	2 days.....	100 grams..	„
Rabbit Bk....	„ „	25 day old *Fusarium moniliforme* var. *subglutinans* culture on Raulin's medium kept at a temperature of 2°C. for last six days. The " mould skin," treated as above, was given to this rabbit (No. 602)	1 day......	60 grams..	„
Rabbit Bl....	„ „	The fluid, from which the " mould skin " drenched to rabbit Bk had been removed, was given to this rabbit (No. 602)	„	120 c.c.....	„
Rabbit Bm...	„ „	25 day old *Fusarium moniliforme* var. *subglutinans* culture on maize kept at a temperature of 2°C. (No. 602)	„	50 grams..	„

EXPERIMENTS WITH FUNGUS-INFECTED FOODSTUFFS CONDUCTED AT ONDERSTEPOORT—(*continued*).

Animal.	Method of administration.	Material fed or drenched.	Period of feeding or drenching.	Total amount fed or drenched.	Result.
Rabbit Bn....	Fed (no additional food given)	30 day old *Fusarium moniliforme* var. *subglutinans* culture on Raulin's medium. The "mould skin," treated as above, was given to this rabbit	1 day.......	60 grams..	Negative.
Rabbit Bo....	,, ,,	The fluid, from which the "mould skin" drenched to rabbit Bn had been removed, was given to this rabbit (No. 602)	,, ...	120 c.c.....	,,
Rabbit Bp....	,, ,,	30 day old *Fusarium moniliforme* var. *subglutinans* culture on maize (No. 602)	,, ...	50 grams..	,,
Rabbit Bq....	,, ,,	35 day old *Fusarium moniliforme* var. *subglutinans* culture on Raulin's medium. The "mould skin," treated as above, was given to this rabbit (No. 602)	,, ...	60 grams..	,,
Rabbit Br....	,, ,,	The fluid, from which the "mould skin" drenched to rabbit Bq had been removed, was given to this rabbit (No. 602)	,, ...	120 c.c.....	,,
Rabbit Bs....	,, ,,	35 day old *Fusarium moniliforme* var. *subglutinans* culture on maize (No. 602)	,, ...	50 grams..	,,
Rabbit Bt....	,, ,,	40 day old *Fusarium moniliforme* var. *subglutinans* culture on Raulin's medium. The "mould skin," treated as above, was given to this rabbit (No. 602)	,, ...	60 grams..	,,
Rabbit Bu....	,, ,,	The fluid, from which the "mould skin" drenched to rabbit Bt had been removed, was given to this rabbit (No. 602)	,, ...	120 c.c.....	,,
Rabbit Bv....	,, ,,	40 day old *Fusarium moniliforme* var. *subglutinans* culture on maize (No. 602)	,, ...	50 grams..	,,
Rabbit Bw....	,, ,,	45 day old *Fusarium moniliforme* var. *subglutinans* culture on Raulin's medium. The "mould skin," treated as above, was given to this rabbit (No. 602)	,, ...	60 grams..	,,
Rabbit Bx....	,, ,,	The fluid, from which the "mould skin" drenched to rabbit Bw had been removed, was given to this rabbit (No. 602)	,, ...	120 c.c.....	,,

EXPERIMENTS WITH FUNGUS-INFECTED FOODSTUFFS CONDUCTED AT ONDERSTEPOORT—(*continued*).

Animal.	Method of administration.	Material fed or drenched.	Period of feeding or drenching.	Total amount fed or drenched.	Result.
Rabbit By....	Fed (no additional food given)	45 day old *Fusarium moniliforme* var. *subglutinans* culture on maize (No. 602)	1 day......	50 grams..	Negative.
Rabbit Bz....	,, ,,	Fungus-infected *Lolium temulentum* Linn. (See Leemann's article on darnel in this Journal.)	38 days.....	3370 grams..	,,
Rabbit Ca....	,, ,,	,, ,, ,,	,, ...	3225 grams..	,,
Pig 789 (7 months old)	A small quantity of milk added	Fungus-infected *Lolium temulentum* Linn. (See Leemann's article on darnel in this Journal.)	3 days.....	4000 grams..	,,
Dog (number lost)	,, ,,	,, ,, ,,	10 days.....	1250 grams..	,,
Pig 791 (8 months old)	,, ,, starved for 18 hours before being fed	45 day old culture of *Fusarium moniliforme* Sh. (var. *subglutinans* Wr. and Rkg.) (No. 222) (on maize)	24 hours.....	2000 grams..	,,
Pig 792 (8 months old)	,, ,,	45 day old culture of *Fusarium moniliforme* Sh. var. *subglutinans* Wr. and Rkg. on barley (No. 222)..	,, ...	2400 grams..	,,
Pig 790 (8 months old)	,, ,,	48 day old culture of *Fusarium graminearum* Schwabe on (barley grain) maize (No. 630)	2 hours....	400 grams..	,,
Pig 789 (8 months old)	,, ,,	42 day old culture of *Fusarium graminearum* Schwabe on barley grain (No. 630)	24 hours...	2400 grams..	,,

From the foregoing table it is evident that, (*a*) a rabbit ingested 1070 gm. of bran very badly infected with a *Rhizopus* sp. (probably "*Rhizopus nigricans*)," a *Fusarium* sp., and bacteria, in the course of sixteen days without suffering any ill-effects; (*b*) rabbits received cultures of "*Fusarium moniliforme*" on maize and on Raulin's medium without any detrimental effects; (*c*) fifty rabbits received cultures of "*Fusarium moniliforme var. subglutinans*" on Raulin's medium, two of these rabbits, which received the washed "mould skin," died with symptoms of paralysis, whilst the others developed no symptoms of poisoning. Twelve rabbits and one pig received cultures of this fungus grown on maize without suffering any ill-effects; the cultures were grown for varying periods and at different temperatures. One pig ingested 2400 grams of a 45 day old culture of this fungus on barley grain without suffering any harmful effects; (*d*) four rabbits received cultures of *Fusarium graminearum* Schwabe on Raulin's medium without developing any symptoms of poisoning; a rabbit and a pig received this fungus grown on maize, and a pig a culture of it grown on barley without any detrimental effects; and (*e*) two rabbits, a pig and a dog, took large amounts of fungus infected *Lolium temulentum* Linn (drabok) (see Leemann's article on this plant appearing elsewhere in this Journal) without suffering any detrimental effects.

Furthermore, each of two rabbits received 240 c.c. of pure Raulin's medium at the rate of 120 c.c. daily without suffering any ill effects.

The fact that the above-mentioned fungi grown on sterilized substrata did not produce any harmful effects upon the experimental animals, does not exclude the possibility of their producing toxic substances when growing together with other fungi and/or bacteria on certain substrata. This point is referred to under "Conditions affecting the toxicity of mouldy foodstuffs."

III.—DISCUSSION.

A.—ARE FUNGUS-INFECTED FOODSTUFFS POISONOUS?

Although most varying results as to the toxic effects of mouldy foodstuffs were obtained by different investigators, it would appear from the literature that foodstuffs infected with the following fungi may at times be harmful to man and animal: "*Mucor mucedo*," "*Mucor racemosus*," "*Mucor stolonifer*," "*Mucor phycomyces*," "*Aspergillus glaucus*," "*Penicillium glaucum*," "*Oidium lactis*," "*Tilletia caries*," "*Ustilago carbo*," "*Ustilago longissima*," "*Ustilago echinata*," "*Puccinia graminis*," "*Puccinia coronata*," "*Puccinia arundinacea*," "*Uromyces apiculatus*," "*Polydesmus exitiosus*," "*Polythrincium trifolii*," "*Epichloë typhina*," "*Actinomyces thermophyles liquefaciens*," "*Endoconidium temulentum*," and "*Fusarium roseum*" Link. ("*Gibberella Saubenettii*").

"*Claviceps purpurea*" (ergot) and "*Claviceps paspali*" have been definitely proved poisonous on more than one occasion. In addition Mitchell (1918) and Theiler (1927 a) have proved beyond doubt that maize infected with *Diplodia zeae* (Schm.) Lev. is poisonous to cattle and sheep.

Recently specimens of fungus infected maize marketed for human consumption were received at Onderstepoort for investigation. These specimens contained up to 12 per cent. of mouldy mealies from which the following organisms were isolated by Drs. B. Doidge and A. C. Leemann of the Division of Botany, Pretoria: "*Penicillium*," "*Aspergillus*," "*Mucor*" and "*Fusarium*." All these fungi are considered harmful to health.

The quantities of these specimens of mealies and mealie meal submitted were too small to allow of feeding experiments being conducted.

The fact that *Diplodia zeae*, which was proved poisonous by Mitchell and Theiler, is frequently found infecting mealies, especially those grown in areas with a high rainfall, warrants special care in the utilisation of fungus-infected maize for household purposes and as a feed for stock.

B.—SYMPTOMS OF POISONING.

The following symptoms have been attributed by different investigators to the ingestion of mouldy foodstuffs : inappetence, nausea, salivation, vomiting, constipation, diarrhoea, colic, tympanites, difficult deglutition icterus, polyuria, cystitis, nephritis, dizziness, staggering, pronounced excitement, marked depression, hyperaesthesia, paralysis of the extremities, hindquarters, tongue, urinary bladder, ears and retina, complete general paralysis, pushing, turning somersaults, bellowing, trembling, convulsions, epileptiform spasms, profuse perspiration, imperceptible pulse, dyspnoea, rapid loss in condition, swelling of the eyelids, abortion, catarrh of the upper air-passages, urticaria, itching, conjunctivitis, vesicular eczema, fever, cyanosis and mummification and necrosis of the prominent parts of the body. The mortality is fairly high.

Apart from these symptoms the spores of rusts and smuts may when inhaled in large amounts cause severe irritation of the mucosa of the buccal cavity and upper air-passages, especially in the human being.

In addition to the above harmful effects attributed to fungi, Jármai (1925) produced visceral and articular gout in geese by force-feeding them with "*Penicillium glaucum*" infected maize.

C.—POST-MORTEM LESIONS.

These are recorded as : gastro-enteritis with haemorrhages and erosions, accumulation of serum in the brain cavities and in the arachnoidal sac, hyperaemia and oedema of the brain and spinal chord, haemorrhagic fluid in the peritoneal cavity, cystitis, nephritis, peritonitis, acute yellow atrophy of the liver bronchitis, haemorrhages in the serous membranes, swelling of the spleen, turbid swelling of the liver, kidneys and myocard and necrosis and mummification of extreme parts of the body.

In some cases the autopsy is negative.

From the above symptoms it would seem that some cases of so-called poisoning with mouldy foodstuffs were probably botulism and parabotulism.

D.—CONDITIONS AFFECTING THE TOXICITY OF MOULDY FOODSTUFFS.

It appears generally accepted that the mycelia and spores of fungi are harmless except for the irritation of mucous membranes caused by them when they are inhaled in large quantities. There are, however, a few exceptions, for example, "*Claviceps purpurea*" and "*Claviceps paspali*," the sclerotia of which are poisonous.

It is considered that the fungi attacking foodstuffs decompose certain constituents of the latter into poisonous substances. Theoretically there are two ways in which fungi could render the substrata toxic, namely, (*a*) by forming poison in their mycelia and spores and retaining it within their own structures, or secreting it into the substrata, or (*b*) causing the formation of poisonous substances by breaking up one or more constituent of the substrata. The latter point will be further elucidated under "The toxic constituents of mouldy foodstuffs."

The following factors may be, and probably are, concerned in the determination of the toxicity of fungus-infected foodstuffs :—

(1) More than one fungus may be necessary to cause the forming of poisonous substances in the substrata, or, a certain fungus may require the presence of some other definite fungus or fungi in order to liberate toxic substances in the substrata.

(2) Fungus-growth (one or more kinds) may have to be associated with the growth of some or other bacterium or bacteria.

(3) Another possibility is that the harmful substances liberated in the substrata by fungus- and bacterial-growth may in themselves be very slightly toxic and that, when present in the same substrata they may, through their synergistic action, become highly toxic.

(4) There is no reason why the different strains of the same fungus should not, as in the case of bacterial strains, vary to a considerable extent in their capacity of producing poisonous substances in the media on which they grow.

(5) The period of growth of the fungus, or of the fungus together with certain bacteria on the substrata may be a limiting factor in the production of toxic substances.

(6) Aeration, moisture content, reaction (whether acid, neutral or alkaline) and temperature of the substrata, most probably influence the liberation of poisonous ingredients.

(7) The constitution of the substrata plays, in some cases at least, an important role in the formation of certain harmful substances, for example, it was found that certain fungi produce more oxalic acid in the substrata when the latter contain calcium than when they are calcium-free. Furthermore, with regard to the production of oxalic acid in fungi cultures, it was found that the origin of the nitrogen present in the media was of great importance.

(8) The individual susceptibility of man and animal, apart from the fact that different classes of animals vary considerably in their susceptibility to poisoning with mouldy foodstuffs, undoubtedly is a factor concerned. Individual susceptibility may in some cases be explained by an inhibited function of the liver and kidneys (cirrhosis, degeneration) and an inflammatory condition of the gastro-intestinal tract at the time of poisoning.

(9) In addition to the above possibilities, which may explain the contradictory information with regard to poisoning with mouldy foodstuffs, I would like to mention that the different investigators, owing to incorrect identifications, may not have been working with the same kind of fungus in spite of the fact that it was recorded as one and the same kind.

E.—THE TOXIC CONSTITUENTS OF MOULDY FOODSTUFFS.

The toxic principles of *Claviceps purpura* (Er.) Tul. (ergot) are accepted to be ergotoxine, ergotinine, tyramin and histamin, and Gieger (1932) succeeded in isolating a very poisonous alkaloid from "*Claviceps paspali.*"

The following poisonous ingredients were, according to the literature quoted, isolated from fungus-infected foodstuffs: glucosidal and alkaloidal substances, biogenic amines (cholin), furfurol, acetic acid, formic acid, phlogosin-like substances, aldehydes, alcohol, acids, trimethylamin, tyramin and ergotin.

Brown (Editorial, 1916), who examined the root of *Mesembrianthemum Mahoni* N.E. Br. found that it contained a mould "*Rhopalocystis nigri*" (="*Aspergillus niger*"), which produced a large amount of oxalic acid when grown on sugar solution.

In the investigations of the toxic constituents of mouldy foodstuffs and artificial cultures of moulds, attention has almost exclusively been paid to the products of decomposition of the proteins present in the substrata. The three substances present in the substrata most likely to be attacked by the fungi are starches, proteins and fats. It seems quite feasible that non- or very slighlty toxic substances will result from the decomposition of fats as these will in the presence of starches and proteins no doubt be very slightly attacked by most fungi.

It would appear that each and every case of poisoning with mouldy foodstuffs will have to be considered on its own merits with regard to the substances responsible for the poisoning. Mouldy foodstuffs of a high protein and low starch content will be liable to form toxic biogenic amines as products of decomposition of the proteins. These toxic biogenic amines may be cholin, acetylcholin, neurin, easily hydrolysable esters of cholin, trimethylamin, tyramin, etc.

On the other hand, starchy foodstuffs (cereals) low in protein will be liable to be decomposed by fungi into the following harmful substances: alcohol, acetaldehyde, ethylaldehyde, formaldehyde, furfurol, acetic acid, formic acid, oxalic acid, etc.

In mouldy foodstuffs containing both starches and proteins the above products of decomposition may by reason of their synergistic action become highly toxic. A further increase in toxicity may be effected by decomposition products caused by bacteria, which are frequently to be found in such foodstuffs.

Fresh hay poisoning is still an unsolved problem. The cumarin contained in fresh hay appears not to be directly concerned in the causation of poisoning.

F.—IMMUNITY TO MOULDY FOODSTUFFS.

(a) *Natural immunity.*

There appears to be an appreciable difference in the susceptibility of the different classes of animals to the effects of mouldy foodstuffs.

Ruminants (cattle and sheep) seem to be more susceptible than horses, mules, donkeys and pigs to "*Diplodia zeae*"-infected maize, whilst it would appear that as a rule pigs, horses, mules, dogs and man are much more susceptible than cattle, sheep, goats and poultry to fungus-infected foodstuffs.

It would thus seem impossible to lay down a general rule as to the relative susceptibility of the different classes of animals to fungus-infected foodstuffs.

(b) *Acquired immunity.*

The available information with regard to an acquired immunity to mouldy foodstuffs is far too incomplete to allow of any definite opinion being expressed The results of immunisation experiments with extracts of "*Aspergillu fumigatus*" were negative.

G.—CAN MOULDY FOODSTUFFS BE UTILISED AS STOCK FEEDS.

This is a question of enormous economic importance to stock owners. While foodstuffs infected with certain fungi may be harmless or even rendered more easily assimilable, owing to the breaking-up by these fungi of otherwise less digestible constituents, it would seem advisable to consider all fungus-infected foodstuffs detrimental to the health of man and animal. On the other hand, such foodstuffs, owing to the fact that they are never, or very rarely, acutely poisonous, may in many cases be utilised as a feed for certain classes of stock provided they do not form more than 10 per cent. of the total ration. However, before proceeding to use mouldy foodstuffs as a general feed, stock owners would be well advised to ascertain the harmfulness or toxicity of such foodstuffs by conducting preliminary feeding experiments on stock of low value. From the foregoing discussion it would appear that as a general rule care should be exercised in the feeding of mouldy foodstuffs to horses and pigs, while ruminants and fowls appear to be less susceptible. In the case of maize infected with *Diplodia zeae* the reverse appears to be true.

IV.—SUMMARY.

(1) For all practical purposes fungus-infected foodstuffs must be considered poisonous until the contrary has been proved by extensive feeding experiments on the different classes of stock.

Diplodia zeae infected mealies have on several occasions been proved poisonous.

(2) It would appear that some cases of food poisoning in animals attributed to fungi were most probably cases of botulism and parabotulism.

(3) Conditions which probably play a role in the formation of toxic substances in mouldy foodstuffs, are discussed.

(4) Reference is made to the possible toxic ingredients of mouldy foodstuffs and more attention than has hitherto been the case, is paid to the possibility of decomposition products of carbohydrates being poisonous.

(5) The problem of fresh hay poisoning is still a mystery.

(6) More attention should be paid to the bacteria (and the products of decomposition caused by them) associated with fungal growth on foodstuffs

(7) There is an appreciable difference in the susceptibility of the different classes of stock to fungus infected foodstuffs.

(8) In the literature consulted no definite information with regard to the development of immunity to mouldy foodstuffs is available

(9) It would appear that harmful mouldy foodstuffs could be fed with impunity to some classes of stock provided they do not constitute more than 10 per cent. of the total ration of such animals.

(10) It is generally accepted that the toxicity of *Lolium temulentum* Linn. (drabok, darnel) to man and animal is due to the grains being attacked by a fungus "*Endoconidium temulentum*"; the active principle is considered to be an alkaloid temulin, which is said to be absent in fungus-free darnel.

(11) Maize and bran very heavily infected with fungi were fed to fowls and rabbits, and also cultures of "*Fusarium moniliforme,*" "*Fusarium moniliforme*" var. "*subglutinans*" and "*Fusarium graminearum*" Schwabbe grown on various substrata were fed and drenched to rabbits and pigs without having deleterious effects.

Large amounts of fungus-infected *Lolium temulentum* Linn. (drabok, darnel) were fed to two rabbits, a pig and a dog without these animals suffering any ill-effects.

V.—ACKNOWLEDGMENTS.

I am indebted to Dr. B. Doidge and Dr. A. C. Leemann, both of the Division of Plant Industry, Pretoria, for the preparation and identification of the fungi cultures used in the experiments conducted at Onderstepoort.

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ADDENDUM.

By Dr. DOIDGE, Principal Plant Pathologist, Division of Plant Industry, Pretoria.

221. *Fusarium graminearum* Schwabe.
222. }
223. } *Fusarium moniliforme* Sh.
224. } var. *subglutinans* Wr. and Rkg.
225. *Fusarium moniliforme* Sh.

Strains 221–225 were isolated from mealie meal supplied by a miller at Bethal, O.F.S., to a firm in the Eastern Transvaal. Natives refused to eat this and said it made them sick. Sample was sent to us by Dr. Murray of the Health Department and under the microscope showed almost as many *Fusarium* spores as starch grains.

F. graminearum is the conidial stage of *Gibberella Saubinettii* and was referred to as *F. roseum* in some of the earlier publications.

284. *F. moniliforme* v. *subglutinans* from maize used for feeding animals at the Zoo: the animals afterwards suffered from paralysis.

(It is not possible that this maize was also infected with *Diplodia zeae*, and that we failed to isolate this fungus from the sample sent?)

550. *F. moniliforme*.—Isolated from green mealie purchased on Pretoria market, March, 1930. The grains were turning light brown and decaying in patches, and some showed a pink discolouration.

602. *Fusarium moniliforme* v. *subglutinans* from grains of maize from Klip River, sent from Pharmacology Department, Witwatersrand University. This is the organism which Prof. Watt worked with, and will probably be mentioned in his book.

622. *Fusarium graminearum* Schwabe. Isolated by Dr. Leemann from maize cob showing moulding and a pinkish discolouration; sent by Prof. Watt.

630. *F. graminearum* Schwabe. Isolated by Capt. J. McDonald, Kenya, organism frequently obtained from maize seeds showing no visible signs of disease.

631. *F. moniliforme* v. *subglutinans*. Isolated from maize by McDonald in Kenya.

632. *F. moniliforme* v. *subglutinans*, from maize seeds germinating on cob sent by Mansveld, Field Husbandry Section from Piet Retief. Isolated by Leemann.

665. *F. moniliforme* v. *subglutinans*, from maize plants from Kinross. Organism isolated from leaf bases, the upper nodes and leaf bases of these plants were badly collapsed.

Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 1, Number 1, 1933.

A Short Summary on our Botanical Knowledge of *Lolium Temulentum* L.

By Dr. A. C. LEEMANN, Division of Plant Industry, Pretoria.

Common names: Afrikaans: Drabok:
English: Darnel. Ivray, Poison Ray Grass;
French: Ivraie (ivre--drunken):
German: Taumellolch (Taumel=giddiness).

Primitive people know their botany well and by some cruel experience were led to distinguish between poisonous and non-poisonous plants. It is therefore in no way astonishing that the toxicity of darnel was well known to the ancients.

The oldest quotation is perhaps that of the Bible, where the description in St. Matthew 13, 25-30, leaves practically no doubt that the darnel was being referred to. It is worth while quoting those lines in full.

" But while men slept, his enemy came and sowed tares among the wheat, and went his way.

" But when the blade was sprung up, and brought forth fruit, then appeared the tares also.

" So the servants of the householder came and said unto him, 'Sir, didst not thou sow good seed in thy field? From whence then has it tares?'

" He said unto them, 'An enemy hath done this.' 'Wilt thou then that we go and gather them up?'

" But he said 'Nay: lest while ye gather up the tares, ye root up also the wheat with them.'

" 'Let both grow together until the harvest: and in the time of harvest I will say to the reapers. Gather ye together first the tares, and bind them in bundles to burn them: but gather the wheat into my barn.' "

This can only refer to the darnel. It is as the householder says hardly distinguishable from wheat in the young stage and it is therefore wise to wait until it is full grown before it is eradicated. The best way of eradicating it is by fire. The above text shows quite clearly how dreaded the weed was: well known to be used as an offence, well known also the time and way to discard it.

In several publications the following quotations from ancient writings are mentioned:—

> The name *Lolium* is mentioned in Vergil and Dioscorides, and seems to derive, according to Guyot, from le celtic *Loloa*.
>
> Ovid says: " Let the field be clear of darnel that weakens the eyes."
>
> In Plautus' comedy the " Braggart Soldier " one servant says to another: " 'Tis a wonder that you are in a habit of feeding on darnel with wheat at so low a price." " Why so." " Because you are so dim of sight."
>
> Then Shakespeare says:—

" Want ye corn for bread?
I think the Duke of Burgundy will fast
Before he'll buy again at such a rate.
'Twas full of darnel: Do you like the taste?"
Henry VI: Act III, Sc. 2.

Then *Gerarde* in 1597, says :—

> "The bread wherein Darnell is, eaten hot causeth drunkeness ; in like manner doth beer or ale where the seed is fallen, or put into the malt."
>
> Sinclair in 1869 holds that "neither birds nor beasts choose this detested food. It is excessively bitter and if ground with wheat into flour and made into bread it renders it not only unpalatable and unwholesome but actually poisonous. But it has from earliest ages borne the name of 'drunken darnel' and there can be no doubt of its deleterious qualities whether in meat or in drink."
>
> Sinclair goes on saying, and his statements remind of the lines quoted from scripture.
>
> "We have often been plagued with darnel ; and the only means we used was enjoining a duty upon the reapers, binders and barn men, to collect it in small bundles for the fire, for which a small reward was given. Its early growth is so much like the wheat plants, that it cannot be weeded out by spud as other weeds are, of course it stands till reaped with the wheat."

Lolium temulentum L, is a native plant of Europe. It is a pest in its native country and has become a pest all over the world. It must have been carried to foreign countries at the same time as wheat. We have attached a photograph of the plant, the specimen being No. 822 of the National Herbarium at Pretoria.

Toxicity is rare in grasses. The toxicity of Lolium cannot be put down with safety to the plant itself.

In the case of Lolium three possibilities must be considered :–

1. Toxicity of plant and grain in themselves.
2. Toxicity of the fungus invading grain and plant.
3. Toxicity from interaction of the plant and the fungus.

We shall endeavour first to consider the question whether the plant in itself is toxic. The plant being a grass there is not much likelihood that it would be so.

Long says : "Before the seeding stage is reached Darnel seems to be quite suitable as food for stock, only the seed or grain being poisonous and this not invariably so." According to some careful investigation by Nestler the fungus can easily be detected in the whole plant from its earliest germination to the adult stage. It seems thus that the plant in itself is not toxic and that we may safely assume that toxicity is only in the seeds.

To ascertain whether the seed in itself is toxic we should attempt to obtain fungus free seeds. Freeman says that in nature there are two races of *Lolium temulentum*, the one is fungus infected and the other is fungus free. He thinks that there is no transference of the fungus from the one to the other. Freeman has, however. failed to prove his statements conclusively, so that they still remain interesting suggestions. From a quotation in Czapek I gather that Hannig has succeeded in producing the two above mentioned races artificially and that the fungus free plants were not toxic. If this experiment is confirmed it would be the ultimate demonstration that the plant in itself is not toxic. It would still remain to be shown that the fungus in itself is toxic, or that the interaction between fungus and plant creates the toxin.

An experimental method that could be followed, although laborious would be the following. To cut seeds in half, to examine one half for the presence of the fungus and to feed the other half. This method would allow with a high degree of probability to test the effect of non-infected material.

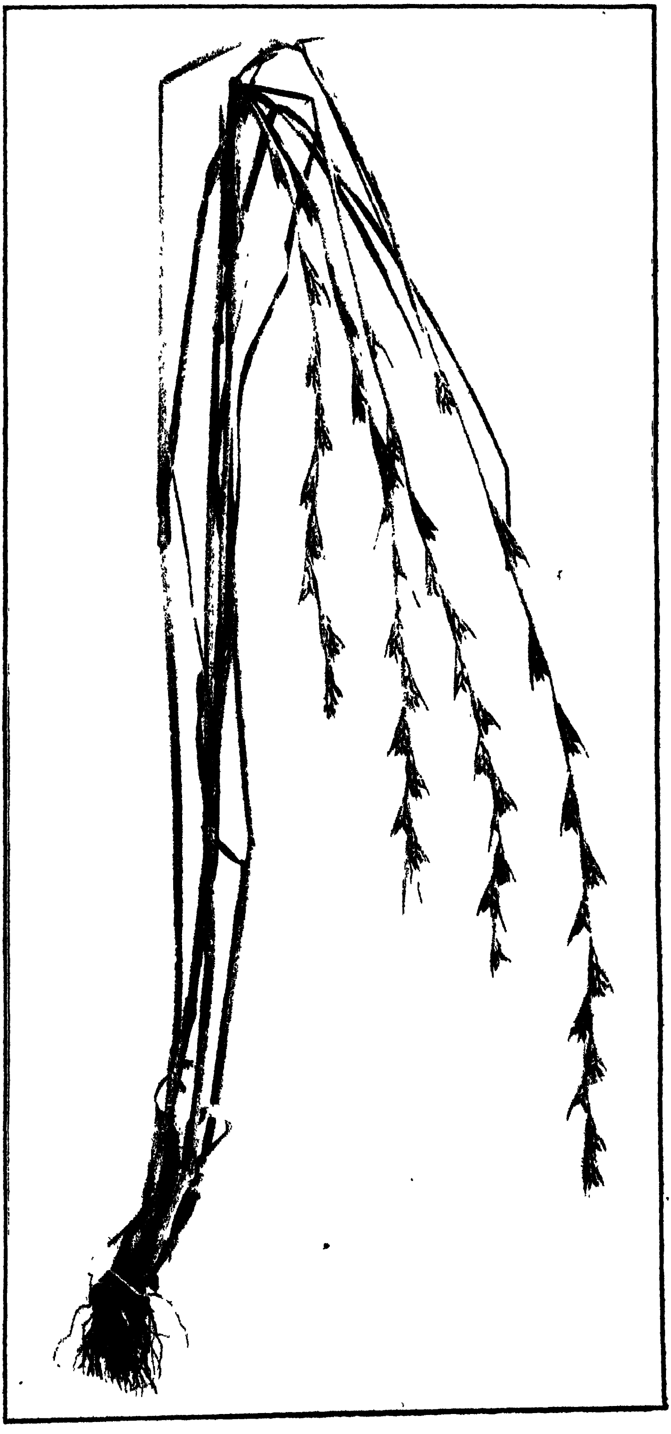

FIG. 1.—*Lolium temulentum* L., National Herbarium 8228. Photo by H. King.

A disturbing factor enters however from the fact that the toxicity seems to be variable. It is reported that in wet seasons the toxicity is more pronounced than in dry seasons. How far that statement is justified I cannot tell. It is well known also that the experiments even during the same season are erratic. A variability of toxicity is quite possible; unfortunately it complicates the whole problem and makes many conclusions uncertain.

We could here refer to the argument put forward by Guerin which although not quite conclusive may yet serve as a useful hint.

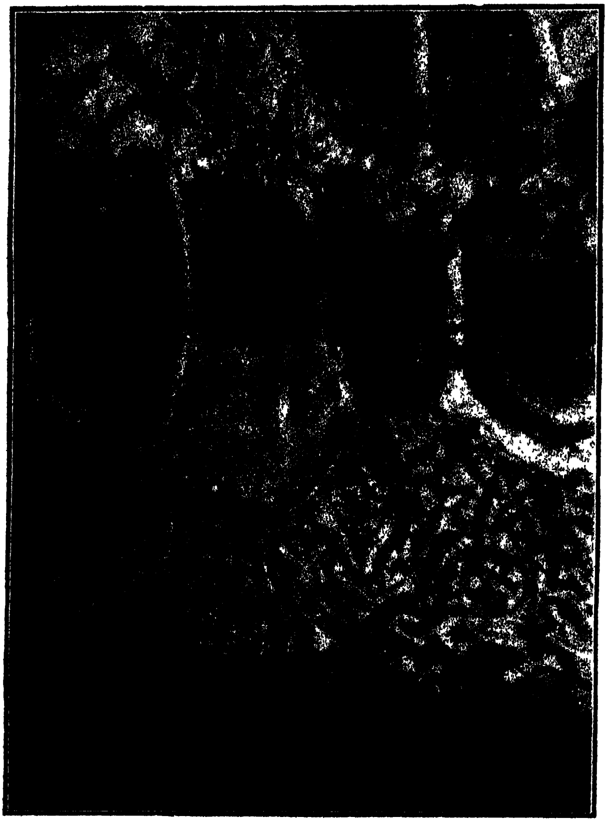

FIG. 2.—*Lolium temulentum*, Mycelium of Fungus above the Aleurone Layer. Photo by A. C. Leemann.

Guerin says: "In forty seeds of most diverse origin the mycelial zone is lacking from but three. This observation had been confirmed in other species of Lolium to wit *L. perenne* L., *L. arvense* With. (var *L. temulentum*), *L. linicola* Sond. It is only exceptional that the first of these contains the parasite. The rest are infected to the same degree as *L. temulentum*. When one observes that the species reported poisonous are the very ones in which we have found the parasite, it seems reasonable to ask whether the temulin of Hofmeister is not a result of the action of the fungus upon the nitrogenous materials in the peripheral region of the seed."

Considering now the fungus infecting the plant, we must admit that we know but little of it. It does not always infect the plant, some of the specimens are absolutely free of fungus.

Vogl seems to be the first who drew attention to the presence of the fungus in the seeds of *Lolium temulentum*. Hanausek, Nestler and Guerin then made a study of it. Guerin states that the nucellus of the ovum is filled with hyphae. With the development of the endosperm the original nucellus is reduced to a small layer at the periphery and the fungus is so to say crowded out. In mature seed the fungus only occurs in the hyaline layer outside the aleurone layer as can be seen from our photomicrograph.

Guyot mentions the interesting fact that G. Lindau in 1904 has detected the fungus in darnel seeds coming from the tombs of one of the Pharaos of the dynasty (2400 B.C.). Nestler has made an extensive study of the fate of the fungus during and after germination. He has detected the fungus right along the stem and followed its path into the seeds. No fructification bodies of the fungus have as yet been found.

Endoconidium temulentum Prill. and Del., has often been considered as the possible parasite. Guerin most emphatically denies that. I feel inclined to follow the idea of Guyot who believes that the above fungus is parasitic on rye and symbiotic in Lolium. The infected rye produces symptoms of intoxication which are very much similar to those in darnel. The suggestion of Guyot should therefore retain our attention. Rye may possibly be a second host to the fungus, a host where it fructifies.

The absence of fructification in the fungus renders determination practically impossible. Nobody has as yet succeeded in making an artificial culture of the fungus. I have also made an attempt on a medium consisting of agar plus ground darnel seeds. But so far nothing but well known saprophytes could be detected in the plates. Nestler has tried a series of other media without result.

The presence of the fungus does not impair germination. This speaks in favour of Guyot's idea of Symbiosis. The infected seeds show an excellent germination. This of course could be brought in parallel with the cases of mycorrhiza where the presence of the fungus is helpful to the metabolism of the plant.

The fungus infecting the darnel has sometimes been considered to be a smut. Although it behaves to a certain extent just like a smut. there is however no definite proof for that supposition. It is therefore best to leave the question entirely open until further evidence permits to decide.

The plant is a nuisance and should be eradicated. It should be picked and burnt *before* its seeds reach maturity.

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Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 1, Number 1, 1933.

Poisoning of Human Beings by Weeds contained in Cereals (Bread Poisoning.)

By D. G. STEYN, B.Sc., Dr. Med. Vet., Veterinary Research Officer, Onderstepoort.

I. INTRODUCTION.

*II. PLANTS DISCUSSED IN THIS ARTICLE :—

A. Borraginaceae.
    Lithospermum arvense L.

B. Caryophyllaceae.
    (*a*) *Agrostemma Githago* L.
    (*b*) *Silene gallica* L.

C. Compositae.
    (*a*) *Centaurea picris* DC.
    x(*b*) *Senecio arenarius* Thunb.
    x(*c*) *Senecio Burchellii* DC.
    x(*d*) *Senecio ilicifolius* Thunb.
    x(*e*) *Senecio isatideus* DC.
    x(*f*) *Senecio laevigatus* Thunb.
    x(*g*) *Senecio rigidus* L.
    x(*h*) *Senecio rosmarinifolius* L. f.

D. Cruciferae.
    (*a*) *Raphanus raphanistrum* L.

E. Euphorbiaceae.
    (*a*) *Euphorbia helioscopia* L.
    (*b*) *Euphorbia peplus* L.
    (*c*) *Ricinus communis* L.

F. Graminae.
    Lolium temulentum L.

G. Leguminosae.
    Vicia sativa L.

H. Polygonaceae.
    Rumex Acetosella L.

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* **Only those plants marked with an *x* are indigenous.**

I. Solanaceae.
  (*a*) *Datura Stramonium* L.
  (*b*) *Datura Tatula* L.

III. Legal Aspect.

IV. Discussion.
  A. Plants Concerned in Bread Poisoning and in Poisoning by other Foodstuffs Cultivated on Lands.
  B. Are *Senecio spp.* Concerned in the so-called "Bread Poisoning" in Human Beings.
  C. Circumstances favouring Bread Poisoning.
  D. Effect of the Process of Preparation of Bread on the Toxicity of Weeds Contaminating the Meal.
  E. The Cause of Death in Senecio Poisoning.

V. Summary.

VI. Acknowledgements.

VII. Literature.

I. INTRODUCTION.

In this article the term "bread poisoning" signifies poisoning caused by the ingestion of bread prepared from wheat contaminated with extraneous seeds. Mention will, however, also be made of poisoning which is liable to occur when other articles of diet, e.g. beans and mealies, become contaminated with extraneous seeds.

The first report of an obscure disease known as "bread poisoning" was made to the Union Government Health Department in 1918 from Albertyn (Willmot and Robertson, 1920), although it had apparently been occurring in the Mossel Bay–George–Riversdale area for quite a number of years. Willmot and Robertson (1920) investigated the disease in the George district and suspected some form of poisoning. As the disease was most prevalent in families belonging to the poorer classes, whose diet consisted mainly of bread, their attention was directed to the wheat and bread consumed by the affected people. Willmot and Robertson at once suspected *Senecio* poisoning, as they knew that certain species of *Senecio* had been established as the cause of the Molteno Cattle Disease, Winton Disease in New Zealand, and Pictou Disease in Nova Scotia, which diseases, in common with the above disease in human beings, show cirrhosis of the liver.

They found *Senecio Burchellii* DC and *Senecio ilicifolius* Thunb. growing on the wheatlands and suggested that at the time of threshing the seeds and other portions of these weeds find their way into the wheat when threshing machines and mills not fitted with efficient winnowing appliances are used.

The ages of the patients examined by Willmot and Robertson varied from eleven to nineteen years and the period that elapsed from the onset of symptoms up to the time of death ranged from fourteen days to two years. The disease is most prevalent amongst young people, although both sexes of all ages may be affected.

The following are the symptoms, post-mortem appearances and histological changes as described by Willmot and Robertson and as verbally communicated to the author by Dr. Shanks of Humansdorp and Drs. van Zyl and G. Muir of Riversdale : A feeling of discomfort over the epigastrium, abdominal pain, nausea, vomiting, especially after meals, the vomit at times containing blood, ascites enlarged liver, apathy, extreme emaciation and diarrhoea with blood in the stools. In one of the Humansdorp cases Dr. Shanks saw very slight yellowish discolouration of the conjunctivae.

Post-mortem appearances.—Enlarged liver with well defined, slightly raised areas of a deeper colour than normal on the surface ; on section these areas, which varied in size from a hazelnut to a walnut, were hyperaemic ; in more advanced cases the liver, which may or may not be enlarged, showed cirrhotic changes and similar sized areas, which were of a lighter colour than the liver substance. In some cases the contents of the stomach, which as a rule was normal in size, were dark-brown. Dark coloured circular spots varying in size from a pin's head to a pea, were noticed on the gastric mucosa. On closer examination these spots were found to be small ulcers the bases of which appeared to be covered with blood.

The stomach may in some cases be dilated. The kidneys showed marked congestion, whilst the remaining organs appeared normal.

Histology of the liver : Recent cases.—The central veins and the capillaries between the hepatic cells are distended : the liver cells are reduced in size, some containing a brown pigment and others fatty particles.

More advanced cases.—These showed the usual round cell infiltration and increased formation of fibrous tissue as seen in cirrhosis of the liver from other causes.

Dr. G. de Kock, Head of the Department of Pathology, Onderstepoort, examined a portion of the liver of one of the suspected " Drabok " cases, which had occurred in the Clanwilliam District (Willmot and Silberbauer, 1931) and found the histological picture very similar to that seen in the livers of horses poisoned with *Senecio spp.* This liver specimen was kindly submitted by the Somerset Hospital, Capetown.

The majority of affected people die unless early treatment is applied and the causative agent removed. Numbers of people who suffer from ascites due to bread poisoning are " tapped " at fortnightly or three-weekly intervals and may recover. It stands to reason that once the liver has been severely damaged over prolonged periods pronounced irreparable changes (e.g. cirrhosis and degeneration), which markedly decrease the functions of this organ, will set in. It is hardly possible that such affected patients, if they recover from an acute attack, will ever return to the normal state of health. What is more, in such patients the function of the liver as chief detoxicator of the system, is very much inhibited, consequently such human beings will be more susceptible to the effects of poisons, both those of exogenous and endogenous origin.

Having suspected poisoning with *Senecio Burchellii* DC. and *Senecio ilicifolius* Thunb. Willmot and Robertson (1920) proceeded to conduct some feeding experiments upon guinea pigs and white rats. The dried flowerheads and " seeds " of the above two *Senecio spp.* were added to the diet of these animals. Willmot and Robertson reported as follows : " All the experimental animals became very emaciated, in spite of the fact that they consumed a normal amount of food. One guinea pig out of twelve under experiment died after feeding for ten weeks on various quantities of dried ground-up seed-heads and tops of a plant identified as "*Senecio ilicifolius*." The post-mortem findings in this guinea pig were almost identical to those in the human subject referred to above, viz: liver mottled, showing to the naked eye well-marked areas of a lighter colour than normal, which on microscopic examination were found to be due to round celled infiltration both intra- and interlobular with the formation of new fibrous tissue. The stomach and upper part of the duodenum contained dark brown fluid (altered blood), and many small specks of blood were found adherent to the stomach wall, chiefly in the neighbourhood of the pyloric end; on washing the blood away, numerous minute ulcers could be made out with a hand lens."

Very similar lesions were found in three white rats which succumbed after having been fed daily for almost four months on three grams of ground-up heads of *Senecio ilicifolius* Thunb. and in one rat which had for three weeks received three grams daily of *Senecio burchellii* DC. The livers of the three rats fed on *Senecio ilicifolius* Thunb. showed cirrhosis, whilst the liver of the rat which had consumed *Senecio burchellii* DC. was congested but not cirrhotic.

The stomach and intestinal contents of all the rats were dark brown and blood-stained and minute pin-point ulcers covered with blood were detectable on the gastric mucosa.

Willmot and Robertson concluded their publication with the following remark:—

"We recognise the incompleteness of our investigations which were unfortunately interrupted by the epidemic of influenza which swept South Africa in 1918. Further enquiry and research are necessary, but it seems desirable to place our preliminary investigations on record."

On 15/2/28 two specimens (53657 and 53658) of wheat and one (specimen 53656) of meal were forwarded to Onderstepoort by the Chief, Division of Plant Industry, Pretoria, with the following remark (Onderstepoort File 144/2282): "These samples have all been examined but there appears to be no trace of *Senecio* present, in fact, the samples of wheat may be said to be clean."

These specimens of wheat and meal were submitted by the Magistrate, Riversdale, as the result of the death of a European girl at Corrente River, Riversdale District, from suspected *Senecio* poisoning. The suspected wheat was grown on the farm where the girl took ill.

Very small amounts of wheat and meal were submitted. Fungus-free seeds of darnel (drabok), barley and oats were found in the two samples of wheat. 200 grams of the meal (specimen 53656) fed to a rabbit caused inappetence, pronounced diarrhoea, extreme weakness, apathy and death within three days after ingestion. The post-mortem revealed marked hyperaemia and slight oedema of the lungs; colourless gelatinous infiltration of the pericardium, mesenterium and wall of the big intestine, the mucosa of which showed numerous small haemorrhages and an acute catarrhal gastro-enteritis. The histological examination of the heart, liver, and kidneys was negative.

150 and 80 grams of wheat (specimen 53657) were fed to two rabbits respectively. Both developed symptoms of severe gastro-intestinal irritation, the former dying within twenty hours and the latter within twenty-four hours after ingestion of the material.

As the samples of wheat and meal caused severe gastro-intestinal irritation and as arsenical poisoning is so common in South Africa the small amount of material that was left of each was examined for the presence of arsenic. The result was negative.

100 grams of wheat (specimen 53658) had no ill-effects on a rabbit.

Each of two control rabbits ingested 450 grams of ordinary wheat in the course of four days without suffering any ill-effects.

Unfortunately the small quantities of the above samples submitted only allowed of these few preliminary experiments being conducted.

More material was requested but with the fresh consignment of wheat no poisoning of rabbits could be induced with the result that the cause of poisoning by the original samples remained a mystery. The following seeds were found in this wheat: *Senecio spp.*, a *Silene sp.*, barley, oats, drabok, a *Rumex sp.*, and *Raphanus spp.*

On 21/4/31 the Principal, Grootfontein School of Agriculture, Middelburg, Cape Province, forwarded to Onderstepoort two samples (405A and 406A) of wheat and two (405B and 406B) of meal, which had been submitted by Dr. Shanks of Humansdorp, who suspected *Senecio* poisoning. One European woman and her two children and four native servants were affected, exhibiting symptoms of gastric disturbances, namely, pain, vomiting and constipation (Onderstepoort File 144/68).

The suspected wheat was produced on the farm where the disease occurred. The samples of wheat, which were too small to allow of any reliable experiments being conducted, contained a large amount of drabok. A small amount of vetch seeds, barley, oats, *Senecio* flowerheads, and seeds of *Silene gallicia* L. were also present.

At the request of the Director of Veterinary Service two bags (specimens 1007 and 1008) of this suspected wheat were forwarded to Onderstepoort for further investigation. In this wheat a large percentage (± 5 per cent.) of drabok *Lolium temulentum* L. was found, as well as a small amount of seed of *Lithospermum arvense* L.

Four rabbits ingested large amounts of this wheat in the course of fourteen days without any deleterious effects. In addition the drabok was sorted out and fed to a rabbit, which consumed 1.550 grams in the course of fifteen days without any ill-effects. 35 grams of the *Lithospermum arvense* L. seeds were drenched to a rabbit with negative results.

It was realised that no progress in the investigations of the problem of bread poisoning could be made by collecting specimens of wheat and meal and submitting them to laborarory tests at the time cases of suspected bread poisoning occur. As bread poisoning is essentially a chronic malady it stands to reason that the particular samples of wheat and meal collected at the time the disease is reported must not necessarily have been concerned in the causation of poisoning. The bread containing the harmful weeds may have been eaten weeks and even months before the disease is reported. The disease is mostly of a chronic nature and in addition disease is very rarely or never reported to the medical people until fairly pronounced symptoms have developed.

Another important fact which renders an investigation into the cause of bread poisoning of very little value at the time such cases are reported is that only some bags of wheat (meal), and of these again some more so than others, are contaminated with the seeds and portions of poisonous weeds.

In the Humansdorp, Riversdale and Clanwilliam districts the author has seen wheat lands infested with *Senecio Burchellii* DC. and *Senecio ilicifolius* Thunb. In quite a number of cases the *Senecio* plants were found growing luxuriantly amongst the wheat in a portion of the lands whilst none or very few of these plants were seen on the rest of these lands.

It was for the above reasons that the importance of investigating the prevalence of weeds on the wheatlands of farms, where suspected bread poisoning occurs, was realised.

The outcome of the co-operation between Sir E. N. Thornton, Assistant Health Officer and Director of Medical Services, Pretoria, and Dr. P. J. du Toit, Director of Veterinary Services, Onderstepoort, in their eagerness to solve this very serious problem, was that the author was requested to visit farms in the Humansdorp, Riversdale, and Clanwilliam districts, where cases of suspected bread poisoning had occurred, in order to study the occurrence and prevalence of poisonous weeds on the wheatlands concerned.

The following weeds were collected by the author on wheatlands in the Humansdorp, Riversdale, and Clanwilliam districts, where cases of suspected bread poisoning had occurred:—

BORRAGINACEAE.

Lithospermum arvense L.

CARYOPHYLLACEAE.

Scleranthus annuus L.
Silene gallica L.

COMPOSITAE.

Crepis polyodon Phillips.
Osteospermum muricatum E. Mey.
Senecio arenarius Thunb. (Clanwilliam only).
Senecio burchellii DC. (not in Clanwilliam).
Senecio ilicifolius Thunb.
Senecio laevigatus Thunb. (Riversdale only).
Senecio rigidus L. (Riversdale only).
Senecio rosmaninifolius L. f. (Riversdale only).

CRUCIFERAE.

Raphanus sp. (pink flower) (Humansdorp only).
Raphanus raphanistrum L.

EUPHORBIACEAE.

Euphorbia helioscopia L. (not in Clanwilliam).
Euphorbia peplus L. (not in Clanwilliam).

GRAMINEAE.

Lolium temulentum L.

LEGUMINOSAE.

Vicia sativa L.

PAPAVERACEAE.

Fumaria officinalis L.

POLYGONACEAE.

Rumex acetosella L.

PRIMULACEAE.

Anagallis arvensis L.

SCROPHULARIACEAE.

Hebenstreitia integrifolia L.

It must be mentioned here that the plant which was identified by Hutchinson (Kew) and Pillans (Bolus Herbarium) as "*Senecio ilificolius*" (teste Dr. J. Muir) and which occurs in the wheatfields in the Riversdale district, appears to be different from the plant of the Humansdorp, George, and Clanwilliam wheat-fields and which was also identified as *Senecio ilicifolius*. Field observations make it difficult to believe that the two are the same species. Specimens of Hutchinson's and Pillans's "*Senecio ilicifolius*" collected by the author on wheatfields at Corrente River, Riversdale, were identified by the Division of Botany, Pretoria, as *Senecio Rehmanni* Bolus.

It is with the Riversdale plant that Willmot and Robertson (1920) conducted their experiments on guinea pigs and white rats (teste Dr. J. Muir).

Muir (1928) made a valuable contribution to the study of *Senecio spp.* and other weeds in relation to the so-called bread poisoning, which occurred to an alarming extent in the Riversdale. George, and Mossel Bay districts.

Muir recorded forty-six species of *Senecio* growing in the Riversdale Division and of these he found the following as weeds on cultivated lands: "*Senecio burchellii*" (abundant), "*Senecio levigatus*" (fairly common), "*Senecio ilicifolius*" (abundant). "*Senecio rosmarinifolius*" (fairly common).

In the course of the above investigation the author made arrangements for large amounts of weeds which were liable to find their way into threshed wheat, to be forwarded to Onderstepoort for experimental purposes, but with a few exceptions, it was a difficult task to obtain any material at all.

The weeds and weed-seeds obtained and experimented with, as well as other plants which may find their way into human foodstuffs, will now be discussed.

II. PLANTS DISCUSSED IN THIS ARTICLE.

A. BORRAGINACEAE.

Lithospermum arvense Linn.

Registered number.—Onderstepoort Spec. No. 6118 (*b*): 19/2/32.

Common and verracular names.—Cromwell-corncockle: clove bush; naaldjie bos.

Origin.—Wheatlands at Modderfontein, Humansdorp.

Parts of plant tested.—"Ripe seeds." When ground these emitted an unpleasant oily smell.

Two rabbits.—Each received per stomach tube a totale amount of 200 grams of these "seeds" in the course of sixty-eight days.

Result.—Negative.

No record of the toxicity of this plant could be found in the leterature. Rosenthal (1862) mentions that the roots, which are of a reddish colour when the plant is immature. are used by the peasant girls of the Northern Countries as a "paint." Huseman and his co-publishers (1882) refer to the red colouring matter which had been isolated by Ludwig and Kromayer from the roots of *Lithospermum arvense* Linn.

The fruit of this plant is used medicinally in gonorrhoea, diarrhoea and as a ecbolic (Dragendorff, 1898). Pammel (1911) states that *Lithospermum arvense* Linn. is very frequently attacked by the fungus *Puccinia rubigo-vera.*

B. CARYOPHYLLACEAE.

(a) *Agrostemma Githago* Linn.

Common and vernacular names.—Koringroos: Corn cockle: Kornrade (German): corn rose; corn campion.

Habitat.—The Flora Capensis (Harvey and Sonder, 1859) describes it as a plant 1 to 2 feet high and having purple flowers; and mentions that it occurs in corn fields and was introduced from Europe. Burtt-Davy (1926) records this plant as having occurred in a patch of European vetches at Groenkloof, Pretoria.

History.—Thunberg (1823) writes: "*Agrostemma githago* inter Triticum satum juxta Fransche Hoek et alibi, ex Europa advena. Versus finem anni floret."

Rosenthal (1862) states that this plant contains a saponin and an amorphous poisonous substance githagin, and that the seed is used as a diuretic, expectorant and anthelmintic and the root as a remedy for haemorrhoids and eczemata. At this early date the seed which frequently finds its way into wheat was known to be detrimental to health. Kobert (1906) refers to this plant at length. In the seed the poison (agrostemmasapotoxin) is situated in the "embroyo and in the cotyledons and not in the seed protein." Horses, bovines, goats, dogs, cats, rabbits, fowls, doves, canaries, and rats are susceptible to this poison when administered per os or subcutaneously. Pigs appear to develop a tolerance to the poison when intitial small doses are given.

The cheaper grades of flour in Europe are frequently adülterated with corn-cockle (Pammel, 1911). Pammel, quoting Millspough, cites a case of two calves which died after having been fed 14¾ oz. of wheaten flour containing 30 per cent. and 45 per cent. of corn-cockle seeds respectively. These seeds cause severe gastro-intestinal irritation and death in ducks and geese. According to Pammel (1911) Chestnut stated that all parts of the plant are poisonous, but the kernel contains most poison. Pammel (1911) says: "The poisoning is generally produced by a poor grade of flour made from wheat containing cockle seeds. Machinery is used to remove these seeds from the wheat, but the difficulty of separating is so great that the result is not entirely accomplished." And again: "Flour containing a smaller amount has often been made into bread and eaten, sometimes with fatal results, the baking not always being sufficient to decompose the poison. The effect may be acute, or, if a small quantity of the meal is eaten regularly, it may be chronic. In the latter case it is sometimes known as a disease under the name of "githagism."

With regard to the amount of *Agrostemma Githago* Linn. seed required to cause poisoning Pammel (1911) again quoting Chestnut, says: "A person eating 1,200 grains of bread made from flour containing only one-half per cent. of corn-cockle seed would consume six grains of cockle seed, an amount which the author believes beyond a doubt to be poisonous in its effects."

Sapotoxin, the toxic principle of corn-cockle, is stated to be only partially destroyed by baking.

Long (1917) mentions that when ground-up with wheat these seeds impart a greyish tint and disagreeable odour to bread made from it. The various investigators have had different results with regard to the effects of corn-cockle seeds on domestic animals. Long, quoting Pesch, says (*a*) that the amount of poison in the seed varies: (*b*) animals develop a tolerance to the poison; (*c*) the susceptibility of animals to the poison varies both with the species and the individual"; (*d*) young animals are more susceptible than older ones; (*e*) "it is believed that rodents and sheep are not susceptible: and, as far as is known, grown cattle are only slightly or not at all affected by the poison: (*f*) calves, swine, horses, and especially dogs, are more or less susceptible": and (*g*) "concerning birds and fowls there is some doubt."

According to Long (1917) the toxic principle is a glucoside which has an acrid taste and which in the course of time has received the following names: Githagin, saponin, agrostemmin, sapotoxin, agrostemma-sapotoxin, and smilacin. Long, quoting Cornevin, gives the following as letha doses of corn-cockle seed per 100 ℔. live weight of animal:

Calf : 0·25 ℔.
Pig : 0·10 ℔.
Dog : 0·90 ℔.
Fowl : 0·25 ℔.

According to Thomson and Sifton (1922) corn-cockle seed has caused so much trouble in the United States of America that in certain States laws have been passed prohibiting the marketing of feeds which are contaminated with even the smallest amounts of these seeds. Referring to these seeds they state that "before the days of modern machinery they often found their way into the flour with disastrous results."

Bernhard-Smith (1923) gives the active principle of corn-cockle as smilacin. De Wilde (1932) followed the saponin content of *Agrostemma Githago* and found that there was an increase in the amount of saponin as the plant ripened. Watt and Brandwyk (1930) also shortly refer to poisoning with corn-cockle in man and animal, quoting work done by Brandl, Brandl and Mayr, Wedekind and Knecke, and Wedekind and Schicke.

Symptoms of poisoning.—The following symptoms are described in the literature: Kobert (1906), Pammel (1911), Long (1917), Fröhner (1919), Thomson and Sifton (1922), and Pugh (1932).

(1) *Human beings: Chronic form.*—It occurs when small amounts of corn-cockle seed are taken over prolonged periods, and is almost invariably the only form met with in human beings. It is characterised by marasmus, weakness, dyspnoea, vomiting, abdominal pains, chronic diarrhoea, and affections of the nervous system.

Acute form.—Intense irritation of the gastro-intestinal tract, nausea, vomiting, headaches, diarrhoea, fever, also vertigo, pains in the spine, impaired locomotion, dyspnoea, dilirium, and coma, which may be followed by death.

The poison is said to be destroyed by good baking of meal contaminated with corn-cockle seed.

(2) *Animals: Chronic form.*—It is rarely met with in animals except the pig. The symptoms closely resemble those met with in chronic corn-cockle poisoning in human beings.

Acute form.—Long (1917) describing the symptoms in the different classes of stock says: "In the horse, if a small quantity only is taken, there is yawning, heavy colic, stamping and evacuation of rather soft faeces. If larger quantities are taken, the symptoms, which commence in about an hour, are salivation, frequent yawning, and turning of the head, colic, pale mucus, hurried and weak pulse, rise in temperature and accelerated respiration. Some time later there are muscular temors succeeded by pronounced rigidity, and the faeces are diarrhoeic and foetid. The animal lies down and getting up is painfull, it falls into a kind of coma, stretches itself to the utmost, and death takes place without convulsions."

In cattle symptoms were observed within one hour after ingestion of corn-cockle seed. The animals showed restlessness, salivation, grinding of the teeth, excitement, colic, coughing, followed within five to eight hours by a period of coma. Furthermore, there is permanent decubitus, repeated foetid diarrhoea, hurried and plaintive respriation, accelerated and progressively weakening pulse, progressive loss of motor and sensory powers and a gradual fall in temperature. Death may occur within twenty-four hours.

Pigs show grunting, salivation, nausea, vomiting, foetid diarrhoea, which may be bloody, clonic muscular spasms, paralysis, coma, and death.

In pregnant animals abortion may occur.

Susceptibility.—Young animals are more susceptible than full grown ones. Sheep, goats, and rodents (rabbits) appear not to be susceptible at all to corn-cockle poisoning; also full-grown cattle are very slightly susceptible. Most susceptible are dogs, horses, pigs, calves, and fowls.

Post-mortem Appearances: (1) *Human beings.*—In the available literature no mention is made of specific lesions, but presumably they will be those of a gastro-enteritis of variable degree and in chronic cases extreme cachexia with the characteristic lesions accompanying it.

(2) *Animals.* Blood is dark and tarry in consistence: furthermore lesions characteristic of severe gastro-intestinal irritation are present; frequently also hyperaemia of the brain and spinal cord and exudates in the cavities of the central nervous system.

Histology.—No information with regard to the histology of the organs of victims of corn-cockle poisoning could be found in the available literature.

Treatment.—Symptomatic treatment (demulcents and stimulants) must be applied; Pammel (1911) mentions that Digitalis antagonises the poisonous action of corn-cockle.

Detection of corn-cockle in meal and gastro-intestinal contents.—Fröhner (1919) describes methods, both botanically and chemically, of detecting corn-cockle in meal and in gastro-intestinal contents.

(*b*) *Silene gallica* L.

Registered number.—Onderstepoort Spec. No. 6118 (*a*), 19/2/32.

Nat. Herb. No. 14270.

Common and verracular names.—Eierbossie: gunpowder weed.

Origin.—Wheatlands at Modderfontein, Humansdorp.

Parts of plant tested.—Ripe seeds and the dry plant in the late seeding stage.

Two rabbits received per stomach tube 235 grams and 940 grams of the ripe seeds (capsules + seeds) in the course of fifty-four days respectively.

Result.—Negative.

Sheep, 31599, received per stomach tube 4,600 grams of the whole plant in the dry state and late seeding stage in the course of eleven days.

Result.—Negative.

History.—The gunpowder weed is recommended as a snake-bite cure (Dragendorff, 1898). The following passage is quoted from Burtt-Davy (1926): "Two cases of horse poisoning, one at Johannesburg in 1909 and one at Bloemfontein in 1913, have been attributed to hay or forage containing a considerable admixture of this weed: in the first case the animal became listless, dull, without evidence of spirit and refusing to eat; in the latter case violent purging and colic resulted."

C. COMPOSITAE.

(*a*) *Centaurea picris* DC.

Registered number.—Onderstepoort Spec. No. 4594 : 8 12/31.

Common names.

Origin.—On cultivated lands, Carolspoort, De Aar.

Parts of plant tested.—Whole plant: dry and in the flowering and early fruiting stage.

The owner of the farm Carolspoort suspected this plant of having caused mortality in sheep grazing on the harvested lands, where there was abundant growth of it. It is also maintained that during harvesting the people, handling crops contaminated with this plant, were affected. No symptoms were however, described.

This plant is also referred to in an article on "Plant poisoning in Stock and the development of Tolerance" published elsewhere in this report.

300 grams of the plant given per stomach tube to sheep on each of two consecutive days invariably caused salivation, hoven, pronounced laboured respiration, groaning, fever, diarrhoea, cyanosis, accelerated pulse, which became progressively weaker, apathy and death within eighteen hours after the second dose had been administered. Cyanosis, asystolic heart and pronounced hyperaemia of the lungs were found at autopsy. In some cases there was, in addition to the described lesions, hydroperitonium, hydrothorax, hydropericardium; dilatation of both heart ventricles: slight oedema of the lungs: acute catarrhal gastro enteritis with numerous haemorrhages in the mucous membrane of the small intestine; pronounced hyperaemia of and haemorrages in the retropharyngeal, bronchial and mediastinal lymph glands, and gegenerative changes in the liver.

Histology.—No specific microscopic lesions were detected in the myocard, liver, kidney, spleen and lymph glands.

In the course of the above experiments it was found that a high degree of tolerance could be induced in sheep by drenching the animals with non-toxic and increasing amounts of the plant.

History.—No reference to the toxicity of *Centaurea picris* DC. is made in available literature. Muir (1928) recorded the fact that *Centaurea melitensis* L. occurs on cultivated lands in the Riversdale district. The latter plant is used as a stomachic (Dragendorff, 1898).

According to Dopheide *Centaurea cyanus* L. (corn-flower) caused complete paralysis in a cow (Fröhner, 1919).

(*b*) *Senecio arenarius* Thunb.

Registered number.—Onderstepoort Spec. No. PN; 15/11/31.

Vernacular name.—Hongerblom.

Habitat.—Cultivated lands, Ou-dam, Clanwilliam.

Repeated attempts were made to obtain some plant material for experimental purposes but without success. No reference to the toxicity of this plant is made in the literature.

(*c*) *Senecio Burchellii* DC.

Registered number.—Onderstepoort Spec. Nos. 4319; (17/11/31) and 114; (6/4/32).

Vernacular name.—Ragwort; sprinkaanbos (in some parts of the South-western Cape Province).

Origin.—Commonage, Humansdorp.

It was realised that in order to obtain the most reliable experimental results, it would be essential to use plants growing on wheatlands on farms where suspected bread poisoning occurs. As attempts to obtain *Senecio Burchellii* DC. from such sources failed, the Extension Officer of the Department of Agriculture stationed at Humansdorp was approached and he kindly collected and forwarded to Onderstepoort the plant used in the under-mentioned experiments.

Part of plant tested.—Whole plant. Dry and in the flowering and " seeding state. The results of the experiments conducted at Onderstepoort are recorded in the following table :—

Experiments with Senecio Burchellii, *VC.*

Animal.	Method of Administration of Plant.	Period of Administration.	Total Amount of Plant Taken.	Loss in Weight of Animal.	RESULT.
Rabbit A..............	Per stomach tube...	11 days........	20 gm. (at rate of 2 gm daily)*	None..........	From the 4th day of experiment there was laboured respiration, apathy, accelerated heart beat and decreased appetite. These symptoms progressed until death on the 11th day of the experiment. *Post-mortem appearances.*—Vessels of conjunctivae injected, hyperaemia and oedema of the lungs, congestion of the liver. *Histology.*—Congestion of the liver, no specific changes in myocard, kidney, and spleen.
Rabbit B..............	Per stomach tube...	172 days.......	772 gm. (i.e. 2 gm. daily for 102 days and 10 gm. daily for last 70 days of the experiment	None..........	This animal appeared to be in normal state of health up to 27·6·32 when it was killed, i.e. 10 days after discontinuation of the experiment. *Post-mortem appearances.*—All organs appear normal. *Histology.*—Nothing abnormal.
Rabbit C..............	Per stomach tube...	174 days... ...	944 gm. (i.e. 4 gm. daily for 104 days and 10 gm. daily for last 70 days of the experiment)	None..	At no time were any symptoms of poisoning noticed except slight dyspnoea and inappetence. Animal was killed within 8 days after discontinuation of the experiment. *Post-mortem appearances.*—All organs appear normal. *Histology.*—Nothing abnormal.
Rabbit D..............	Per stomach tube...	95 days........	820 gm. (i.e. 10 gm. daily for 95 days)	28 per cent.....	Progressive dyspnoea, cachexia, inappetence, accelerated heart beat and apathy set in from the 6th day until death supervened on the 95th day of the experiment. At times a slight yellowish discolouration of the conjunctivae, which were markedly injected, was seen. *Post-mortem appearances.*—Anaemia, cachexia, hydroperitoneum, pronounced oedema and hyperaemia of the lungs, heart in systole, atrophy, congestion and cirrhosis of the liver, chronic catarrhal gastritis. *Histology.*—Liver: Hyperaemia, atrophy, interstitial hepatitis.
Rabbit E..............	Per stomach tube...	122 days.......	1,810 gm. (i.e. 40 gm. daily for 28 days and 10 gm. daily for last 94 days of experiment	None..........	This animal developed no symptoms of poisoning. Killed within 14 days after discontinuation of experiment. *Post-mortem appearances.*— *Histology.*—Nothing abnormal.
Dog 1044 (6 months old) (mixed breed)	Per stomach tube...	92 days........	400 gm. (i.e. 5 gm. daily for 92 days)	None..........	In the course of the experiment and up to the time of publishing this article (that is six months after the discontinuation of the experiment) no symptoms of poisoning were noticed in this animal.
Dog 1045 (10 months old)	Per stomach tube...	92 days........	1,350 gm (i.e. 15 gm. daily for 62 days and 20 gm. daily for 30 days	None..........	In the course of the experiment and up to the time of publishing this article (that is six months after the discontinuation of the experiment) no symptoms of poisoning were noticed in this animal.
Sheep 31599 (6 tooth)....	Per stomach tube...	14 days........	5,200 gm (i.e. 400 gm. daily)	None..........	In the course of the experiment and up to the time of publishing this article (that is six months after the discontinuation of the experiment) no symptoms of poisoning were noticed in this animal.

* Except Sundays.

Five rabbits, two dogs, and one sheep were used in this experiment. Of these animals only two rabbits died of which one (rabbit D) may possibly have succumbed to the effects of *Senecio Burchellii* DC., although this appears doubtful in view of the results obtained with *Senecio ilicifolius* Thunb. and *Senecio isatideus* DC. The experiments with the two latter plants proved that rabbits are much less susceptible than dogs to *Senecio* poisoning.

It is unfortunate that the experiments with *Senecio Burchellii* DC. could not be conducted with specimens of the plant collected on lands of farms where cases of suspected bread poisoning have occurred, as cultivation, fertilisation and type of soil may influence the toxicity of plants to a considerable extent.

It was attempted to drench dogs with larger quantities of the plant than those recorded in the above table, but vomiting invariably occurred after drenching young dogs with amounts exceeding 20 grams.

Histology.—Rabbit D, which had received 10 grams of the plant daily for 95 days, showed hyperaemia and atrophy of the liver and an interstitial hepatitis. No specific lesions were detectable in the livers of the remaining four rabbits.

History.—Chase, Verney, and Robertson were the first investigators to prove *Senecio Burchellii* DC., and *Senecio latifolius* DC. (now shown to be *S. retrorsus* DC) poisonous to horses. They attributed "dunsiekte" in horses to poisoning with these two *Senecio spp.* [Theiler, 1918 (*a*)]. Watt (1909) isolated two alkaloids (Senecifolin and Senecifolidine) from *Senecio retrorsus* DC. (then incorrectly named *Senecio latifolius* DC).

Cushny (1911), who experimented with these two alkoloids says: "The symptoms and post-mortem findings in animals poisoned with these alkaloids resemble so closely those described by Gilruth, Chase, Pethick and others, in cattle and horses, that there can be no question that the cause is the same in each and that Pictou, Winton, or Molteno disease is really more or less chronic poisoning with Senecio alkaloids."

Theiler [1918 (*a*) and 1918 (*b*)] discussed at length Senecio poisoning in horses.

Willmot and Robertson (1920) fed one white rat for three weeks with *Senecio Burchellii.* On post-mortem this animal showed a congested but not cirrhotic liver (see Introduction).

Van Es and his co-publishers (1929) found "*Seneciofremonti*" and "*Senecio Riddellii*" poisonous to horses.

Craig, Kearney, and Timoney (1930) refer to the toxicity of *Senecio latifolius* DC. and *Senecio Burchellii* DC. (South Africa), and *Senecio Jacobea* L. (Ireland, Great Britain, Europe, New Zealand, Canada, and Asiatic Russia).

Jalving (1930) succeeded in producing liver lesions in calves by feeding them on "*Senecio aquaticus*" and "*Senecio Jacobea.*"

Further experiments were conducted by De Kock, Du Toit, and Steyn (1931) in order to ascertain whether "dunsiekte" in horses and Senecio poisoning were identical.

Ewart (1931) writes that Molteno disease due to prolonged feeding with '*Senecio latifolius*" may be caused by the saponin content of this plant.

On the farm Modderfontein, Humansdorp district, where several cases of suspected bread poisoning had occurred, the author found *Senecio Burchellii* DC. growing luxuriantly amongst the wheat in a corner of the land concerned. Only two specimens of *Senecio ilicifolius* Thunb. were present on this land.

The owner of this farm requested me to examine a horse, which had been ailing for the past few months. This animal exhibited symptoms which could not be distinguished from those produced at Onderstepoort by feeding and drenching horses with *Senecio retrorsus* DC. (*Senecio latifolius* DC., now *S. retrorsus* DC.) and *Senecio isatideus* DC.

This horse was stabled and allowed to run in a small land near the homestead. The land was found to be heavily overgrown with *Senecio Burchellii* DC.

Manske (1932) has isolated the alkaloid retrorsine from "*Senecio retrorsus*" which he obtained from South Africa and the alkaloid jacobine from "*Senecio jacobaea*." Of great interest is that Manske found the former Senecio to contain 1·3 per cent. of alkaloid and the latter only 0·04 per cent. of alkaloid.

(*d*) *Senecio ilicifolius* Thunb.

Registered number.—Onderstepoort Spec. No. 6179: 23/2/32.

Nat. Herb. No. 14269.

Vernacular names.—Sprinkaanbos: kovanna (guano-) bos (Clanwilliam).

Origin.—D. Botha. George.

The plant material was collected from a land on a farm where cases of suspected bread poisoning had occurred.

Parts of plant tested.—Whole plant; dry and in the flowering and "seeding" stage.

The experiments conducted at Onderstepoort are recorded in the following table:—

TABLE 2.

Experiments with Senecio Ilicifolius *Thunb.*

Animal.	Method of Administration of Plant.	Period of Administration.	Total Amount of Plant Taken.	Loss in Weight of Animal.	RESULT.
Rabbit F..............	Per stomach tube...	4 days.........	4 gm. (at rate of 1 gm. daily)	None..........	On the 4th day of the experiment the animal showed dyspnoea accelerated heart beat, and inappetence. It became very restless, pushed the head violently and repeatedly against the cage walls and died on the same day during such a fit. *Post-mortem appearances.*—Cyanosis, pronounced dilatetion of both heart ventricles, hyperaemia and emphysema of lungs, hyperaemia of liver, which showed a marked resemblance to that in acute senesiosis in horses, stomach: Wall covered with thick greyish mucus and a dark reddish brown substance, which spectroscopically proved to be changed blood, in addition the stomach showed fairly extensive ulceration. *Histology.*—Liver: Slight hyperaemia and multiple localised necrosis. Myocard: Spleen and kidney showed no lesions.
Rabbit G..............	Per stomach tube...	4 days.........	8 gm. (at rate of 2 gm. daily)	2·3 per cent....	Died on the 4th day of the experiment. Symptoms and post-mortem appearances as in rabbit F, with the exception of ulceration of the stomach. *Histology.*—Liver: Fatty changes, hyperaemia, central necrosis and several neutrophiles present. Spleen, myocard. and kidneys showed no lesions.
Rabbit H..............	Per stomach tube...	121 days.......	1,040 gm. (at rate of 10 gm. daily) *	—	Developed no symptoms of poisoning. Killed 10 days after discontinuation of the experiment. *Post-mortem appearances.*—All organs appeared normal. *Histology.*—Nothing abnormal.
Rabbit J..............	Per stomach tube...	120 days.......	554 gm. (at rate of 1 gm. daily for 62 days and 10 gm. daily for 58 days)	—	No symptoms of poisoning developed. Killed on the day the experiment was discontinued. *Post-mortem appearances.*—All organs appeared normal. *Histology.*—Nothing abnormal.
Rabbit K..............	Per stomach tube...	120 days.......	608 gm. (at rate of 2 gm. daily for first 62 days and 10 gm. daily for last 58 days	—	No symptoms of poisoning appeared. Killed 14 days after discontinuation of the experiment. Animal gained in weight. *Post-mortem appearances.*—All organs appeared normal. *Histology.*—Nothing abnormal.
Rabbit L..............	Per stomach tube...	120 days.......	716 gm. (at rate of 4 gm. daily for first 62 days and 10 gm. daily for last 58 days	—	Apart from pronounced loss in weight and dyspnoea, no symptoms of poisoning were exhibited. Killed within 14 days after discontinuation of the experiment. Animal gained in weight. *Post-mortem appearances.*—All organs appeared normal. *Histology.*—Nothing abnormal.

TABLE 2.—(*continued*).

Animal.	Method of Administration of Plant.	Period of Administration.	Total Amount of Plant Taken.	Loss in Weight of Animal.	RESULT.
Dog 1042 (12 months old) (mixed breed)	10 gm. of ground-up plant mixed in food daily	90 days........	Approximate 180 gm........	27 per cent.....	Diarrhoea was noticed on the 27th day of the experiment, then followed progressive loss in condition, anaemia, apathy, inappetence, repeated vomition; *N.B.*—At no time was icterus seen. On the 91st day of the experiment the animal was found prostrate, vomiting foaming mucus these were chronic spasms of the front legs and at intervals of a few seconds clonic spasms of the neck causing the head to be drawn right in between the front legs, the cornea reflex was decreased. Death occurred the same day. *Post-mortem appearances.*—Pronounced cachexia and anaemia, marked hydroperitoneum (150 c.c.), pronounced cirrhosis (atrophic) and degeneration of the liver (not pigmented), extensive ulceration of the gastric mucosa, especially in the pyloric portion with haemorrhages, dark reddish brown mucous substance in the stomach. *Histology.*—Liver: Cirrhosis and degeneration. Blood and spleen smears.—Negative.
Dog 1043 (9 months old) (mixed breed)	Per stomach tube and 10 gm. of ground-up plant mixed in food daily	45 days........	Approximate 480 gm. (at rate of 10 gm. per stomach tube daily and approximate 90 gm. taken with the food)	37·5 per cent..	Diarrhoea appeared on the 16th day of the experiment. There was progressive inappetence, cachexia, dyspnoea and apathy. Diarrhoea was followed by constipation and this again by diarrhoea, pronounced injection of conjunctivae and eye ball vessels, pulse weak and accelerated, fever, staring coat, retching, vomition, extreme cachexia and weakness; on the 45th day of the experiment there was slight general icterus, which was very pronounced on the day of death, which occurred on 47th day of the experiment. *Post-mortem appearances.*—Pronounced general icterus, anaemia, extreme cachexia, blood very dark and not coagulated, slight hyperaemia of the lungs, diastolic heart, pronounced hydroperitoneum (185 c.c.), pronounced cirrhosis and degeneration of the liver, pronounced subacute catarrhal gastritis, pyloric portion of the stomach covered by a dark reddish brown mucous substance, which spectroscopically and chemically proved to be changed blood, a fair number of pin-point ulcerations were present on the gastric mucosa, subacute catarrhal enteritis, the intestine containing a large amount of dark reddish brown mucous substance, which proved to be changed blood. Straw and sand in stomach and intestine. *Histology.*—Liver: Cirrhosis and degeneration. Blood and spleen smears.—Negative.

TABLE 2—(*continued*).

Animal.	Method of Administration of Plant.	Period of Administration.	Total Amount of Plant Taken.	Loss in Weight of Animal.	RESULTS.
Dog 1047 (15 months old) (mixed breed)	Per stomach tube and 5 gm. of ground-up plant mixed in food daily	82 days........	Approximately 435 gm. (at rate of 5 gm. per stomach tube daily and approximately 80 gm. taken with the food)	18·7 per cent..	Diarrhoea appeared on the 19th day of the experiment. The symptoms markedly resembled these described in dog 1043. The conjunctivae showed a slight yellowish discolouration on the 79th day of the experiment. There was a pronounced general icterus on the day of death, which occurred on the 83rd day of the experiment. *Post-mortem appearances.*—Intense general icterus, cachexia, pronounced hydroperitoneum (200 c.c.),marked dilatation of both heart ventricles, tumor splenis with extensive haemorrhages, pronounced pigmentation (dark orange-yellow), degeneration, swelling and cirrhosis of the liver, haemorrhages in periportal lymph glands, straw and sand in stomach, haemorrhages in and slight ulceration of the pyloric portion of the stomach, subacute catarrhal enteritis, extensive haemorrhage into mucous membrane of colon. *Histology.*—Liver : Cirrhosis and degeneration. Blood and spleen smears.—Negative.
Dog 1051 (12 months old) (mixed breed)	5 gm. of ground-up plant mixed in food daily	51 days........	30 gm...?................	—	Feeding of plant was discontinued on the 51st day of the experiment, when the animal had developed distinct symptoms of Senecio poisoning (loss in weight. diarrhoea, constipation. inappetence. and pronounced apathy), in order to study the further course of the disease. Improvement in the condition of the animal set in within 14 days and the animal appeared to be in normal health within 10 weeks after the discontinuation of feeding *Senecio ilicifolius* Thunb.

Six rabbits and four young dogs were used in the above experiments.

Rabbits.—Rabbits F and G which had received 4·0 and 8·0 grams of plant respectively, died on the fourth day of the experiment with symptoms of dyspnoea, inappetence, heart weakness and violent excitement (probably due to asphyxia). The following lesions were found at post-mortem: General cyanosis, dilatation of the heart ventricles; hyperaemia and emphysema of the lungs; hyperaemia and degenerative changes in the liver, and ulceration of the gastric mucosa in one rabbit, (F). The stomach of rabbit F contained a fair quantity of a dark reddish brown mucous substance, which spectroscopically and chemically proved to be changed blood.

Histology.—The liver of rabbit F showed slight hyperaemia and multiple localised necrosis whilst fatty changes, hyperaemia, central necrosis and several neutrophiles were present in the liver of rabbit G. No microscopical lesions were detectable in the spleen, kidney and myocard of these rabbits.

The remaining four rabbits, which developed no symptoms of poisoning having after received 1,040 grams, 554 grams, 608 grams, and 716 grams respectively over prolonged periods, were killed within different periods, varying up to fourteen days, after discontinuation of the experiment. No macroscopic and microscopic lesions were present at post-mortem.

Dogs.—All four dogs, two of which were drenched and also received the plant in their food while the other two received the plant in their food only, developed symptoms of poisoning. Three of these dogs died after having showed the following symptoms: Progressive cachexia, general weakness, inappetence, apathy, anaemia, repeated vomition, diarrhoea, constipation, weak and accelerated pulse, allotriophagia, dyspnoea, general icterus not in dog 1042. In addition to these symptoms dog 1042 showed clonic spasms of the front legs and, at intervals of a few seconds, clonic spasms of the neck.

Post-mortem Appearances.—Pronounced general icterus (dogs 1047 and 1013), anaemia, extreme cachexia, pronounced hydroperitoneum, dilatation of both heart veortricles, hyperaemia of the lungs, pronounced cirrhosis, degeneration and pigmentation of the liver (*N.B.*—the liver of dog 1042 showed no pigmentation), subacute catarrhal gastro-enteritis, haemorrhages in and ulceration of the mucosa of the pyloric portion of the stomach, the stomach and intestine contained a dark reddish-brown mucous substance, which spectroscopically and chemically proved to be changed blood, in addition sand and straw were found in the gastro-intestinal tract, extensive haemorrhage into the mucosa of the colon.

Histology.—This aspect of seneciosis is being fully discussed by Dr. G. de Kock, head of the Department of Pathology, Onderstepoort Laboratories, in a paper dealing with the pathology of Senecio poisoning. It, therefore, suffices to state here that chonic *Senecio* poisoning in dogs is characterised by pronounced cirrhosis (atrophic) and degeneration of the liver.

From the above table it is evident that rabbits are much less susceptible than dogs to poisoning with *Senecio ilicifolius* Thunb. 180 grams of this plant taken by dog 1042 over a period of ninety days sufficed to cause death, whilst rabbits receiving quantities of plant varying from 554 grams to 1,040 grams developed no symptoms of poisoning.

In view of the insusceptibility of rabbits H, J, K, and L it is doubtful whether rabbits F and G succumbed to Senecio poisoning unless we except an enormous hypersusceptibility in these two cases.

It appears that dogs receiving very small quantities of the plant over prolonged periods are not liable to develop general icterus, while this symptom is very pronounced in dogs, receiving larger amounts of the plant over shorter periods.

Young dogs which have developed fairly distinct symptoms of *Senecio ilicifolius* Thunb. poisoning, will recover provided the feeding of the plant is discontinued and the liver has not been damaged beyond repair. It is, however, doubtful whether such damaged livers will completely recover as a certain amount of cirrhosis is bound to persist.

History.—Willmot and Robertson (1920) produced liver lesions in one of twelve guinea pigs and three white rats which had been fed with "*Senecio ilicifolius*" for almost four months (see Introduction). As explained previously Dr. J. Muir verbally informed the author that Willmot and Robertson conducted their experiments with "*S. ilicifolius*" collected on wheatlands at the farm Corrente River, Riversdale district. The author collected specimens of this plant in the presence of Dr. Muir (Riversdale). These specimens were identified by the Division of Botany, Pretoria, as *Senecio Rehmanni* Bolus. (N.H. No. 14267).

Willmot and Silberbauer (1931) describe four cases of ascites in male European adults and state "in view of the absence of gastric disturbance and pain associated with Senecio poisoning, Drabok poisoning was almost certainly the cause." These cases occurred on the farm Palmietfontein (Oudam), Clanwilliam district, and will be referred to more fully under *Lolium temulentum* L. (Drabok) and under "Discussion."

Muir (1931), who was invited by the Editor of the Journal of the Medical Association of South Africa to add a note to the article on "Darnel (*Lolium temulentum*) or Drabok Poisoning" by Willmot and Silberbauer (1931), writes: "I will merely note in passing that partial reliance by Drs. Willmot and Silberbauer on the absence of pain for the diagnosis of drabok poisoning is in conflict with the extract given above. It is further the experience of my colleague, Dr. J. W. van Zyl, District Surgeon here, that certain cases of *Senecio* disease occur where pain is not a prominent feature or is even absent."

In November, 1931, the author visited the above farm Palmietfontein (Oudam), Clanwilliam district, where the cases of suspected drabok poisoning (Wilmot and Silberbauer, 1931) have occurred. On inspecting the land where the suspected wheat was grown, *Senecio ilicifolius* Thunb., locally known as kovanna-(guano)-bossie(bos) was found growing quite abundantly in one corner, whilst only a few specimens of this plant were found on the remaining portion of this land. In addition a neighbouring fallow land was overgrown with *Senecio ilicifolius* Thunb. The owner (Mr. Smit) of the farm promised to forward two bags of this plant to Onderstepoort for experimental purposes, but in spite of repeated reminders the promise was not fulfilled.

As deaths in mules had been reported by Mr. van Zyl, Paardekop, a farm adjoining Palmietfontein, it was decided to investigate the cause of the disease. Twelve mules and one horse had died in the course of a few months after having shown symptoms (described by Mr. van Zyl) very closely resembling those produced at Onderstepoort in horses by feeding and drenching them with *S. retrorsus* DC. (*Senecio latifolius* DC.) and *Senecio isatideus* DC. There was abundant growth of *Senecio ilicifolius* Thunb. on the lands where the oats fed to the above affected animals were grown and Mr. van Zyl admitted that some of the sheaves of the suspected oats were heavily contaminated with this plant.

(e) *Senecio isatideus* DC.

Registered number.— Onderstepoort Spec. No. 5789 : 11/2/32.
Nat. Herb. No. 10848.

Common and vernacular names.—Dan's cabbage : Inkanga (Zulu) ; Poisonous ragwort.

Origin.—Greytown, Natal.

Parts of plants tested.—Whole plant ; dry and in preflowering stage.

Senecio isatideus DC. is of widespread occurrence in South Africa, namely, from Uitenhage through the Eastern Province and Natal into the Transvaal. It was, therefore, thought advisable to discuss here also the toxicity of this plant as it is quite possible that it may find its way on to wheatlands and in this way become a menace to the health of human beings.

In the following table are recorded the results of experiments conducted at Onderstepoort upon dogs and rabbits :—

TABLE 3.

Experiments with Senecio Isatideus *DC.*

Animal.	Method of Administration of Plant.	Period of Administration.	Total Amount of Plant Taken.	Loss in Weight of Animal.	RESULT.
Rabbit M..............	**Per stomach tube**...	78 days........	134 gm. (at rate of 2 gm. daily) *	17 per cent.....	Progressive inappetence, anaemia, dyspnoea, apathy, weak and accelerated heart beat and cachexia until death on the 134th day of the experiment. *Post-mortem appearances.*—emaciation, flabby heart, hydroperitoneum, hyperaemia and oedema of the lungs, extensive haemorrhages into mesentery and subserosal tissues of big intestine, ulceration of gastric mucosa, atrophic cirrhosis of the liver. *Histology.*—Liver: Hyperaemia, multiple necrotic areas. Myocard spleen and kidney showed no lesions.
Rabbit N..............	**Per stomach tube**...	13 days........	48 gm. (at rate of 4 gm. daily)	8 per cent......	Symptoms like those seen in Rabbit M, death occurring on the 13th day of the experiment. *Post-mortem appearances.*—Pronounced hydroperitoneum, flabby heart, liver swollen, nugmeg-like in structure. *Histology.*—Liver: Slight hyperaemia, multiple necrosis, slight increase in connective tissue. Myocard, spleen, and kidney showed no lesions.
Rabbit O..............	**Per stomach tube**...	2 days.........	20 gm. (at rate of 10 gm. daily)	None..........	Apathy, inappetence, laboured respiration and death on the 3rd day of the experiment. *Post-mortem appearances.*—Cyanosis, slight hydroperitoneum, hydrothorax, pronounced hyperaemia of lungs, flabby heart, dark reddish brown (changed blood) mucus on gastric mucosa, liver swollen and dark red, spleen swollen. *Histology.*—Liver: Slight hyperaemia, no specific changes. Hyperaemia of kidney and spleen.
Rabbit P..............	**Per stomach tube**...	34 days........	300 gm. (at rate of 10 gm. daily)	22 per cent.....	Progressive apathy, inappetence, anaemia, dyspnoea, and cachexia until death on the 34th day of the experiment. *Post-mortem appearances.*—Hyperaemia and oedema of the lungs, very flabby heart, subserosal haemorrhages along gastro-intestinal tract, large number of small dark reddish brown spots and ulcers on gastric mucosa, liver: Consistence very firm and of a diffuse light greyish colour. *Histology.*—Nothing abnormal.

* Except Sundays.

TABLE 3—(*continued*).

Animal.	Method of Administration of Plant.	Period of Administration.	Total Amount of Plant Taken.	Loss in Weight of Animal.	RESULT.
Dog 1041 (8 months old) (mixed breed)	Per stomach tube and 5 gm. of ground-up plant in the food daily	20 days.....	Approximately 110 gm. (at rate of 5 gm. per stomach tube daily for 20 days and approximately 1 gm. taken with the food daily)	37 per cent...	Inappetence, apathy, alternating diarrhoea and constipation, ulcerative stomatitis, pronounced progressive emaciation, weak and accelerated pulse, dyspnoea, prostrate for last four days, showing regular spasmodic jerks of all four legs, the animal died in a comatose state on the 20th day of the experiment. *Post-mortem-appearances.*—Ulcerative stomatitis, extreme cachexia, pronounced degenerative changes in the liver, multiple hydronephrosis and cirrhosis of the kidney, ulcerative gastritis, dark brown mucous substance in small intestine. *Histology.*—Liver: Degenerative changes. Spleen of blood smears.—Negative.
Dog 1050 (8 months old) (mixed breed)	5 gm. of the ground-up plant mixed in the food daily	82 days........	Approximately 50 gm...	—	Symptoms of poisoning (except icterus) very similar to those described in Dog 1048 commenced on the 11th day of the experiment. Feeding of the plant was discontinued on the 82nd day of the experiment, when the animal had lost 30 per cent. of its weight and appeared very ill. Improvement was discernible within three weeks after discontinuation of the feeding of the plant. Three months later the animal appeared to be in normal health again.
Dog 1046 (12 months old) (mixed breed)	5 gm. of the ground-up plant mixed in the food daily	77 days......	Approximately 50 gm.......	58 per cent....	Symptoms as those described in Dog 1048, with the exception that icterus never seen in dog 1046. The animal died on the 78th day of the experiment. *Post-mortem appearances.*—Extreme cachexia and anaemia, *N.B.* no icterus, hydroperitoneum, slight hyperaemia of lungs, extensive ulceration of pyloric portion of stomach, some ulcers having healed again; subacute catarrhal enteritis; pronounced cirrhosis (atrophic) of liver (no pigmentation); straw and sand in gastro-intestinal tract. *Histology.*—Liver: Pronounced cirrhosis.

TABLE 3—(*continued*).

Animal.	Method of Administration of Plant.	Period of Administration.	Total Amount of Plant Taken.	Loss in Weight of Animal.	RESULT.
Dog 1048 (12 months old) (mixed breed)	Per stomach tube and 10 gm. of ground-up plant mixed in the food daily	31 days........	Approximately 332 gm. (at rate of 10 gm. per stomach tube daily and 2 gm. taken with the food daily)	25 per cent ..	Inappetence, yawning, progressive apathy and cachexia, alternating diarrhoea and constipation, dyspnoea, general icterus appeared 2 days before death, weak and accelerated pulse, retching and vomiting at intervals, death, preceded by a comatose state, occurred on the 31st day of the experiment. *Post-mortem appearances.*—Intense general icterus, marked hydroperitoneum, hyperaemiae of the lungs, intense pigmentation, degeneration and cirrhosis of the liver, which was extremely firm in consistence, haemorrhages in and ulceration of gastric mucosa, acute catarrhal enteritis, which contained a large amount black blood-like mucous substance, which chemically and spectroscopically proved to be changed blood, straw and sand in gastro-intestinal tract. *Histology.*—Liver: Cirrhosis and degeneration.
Dog 1049 (14 months old) (mixed breed)	10 gm. of ground-up plant mixed in food daily	42 days........	Approximately 84 gm......	45·1 per cent	The symptoms resembled those in dog 1048. Slight icterus appeared on the 56th day of the experiment and increased in intensity up to the time of death, which occurred on the 62nd day of the experiment. *Post-mortem appearances.*—Intense general icterus; pronounced hydroperitoneum (500 c.c.); slight hyperaemia of the lungs; pronounced pigmentation (dark-orange yellow), degeneration, swelling and cirrhosis of the liver; dark greenish, mucous, flocculent bile; hyperaemia and ulceration of the pyloric portion of the stomach with haemorrhages, subacute catarrhal enteritis with a dark reddish brown mucous substance in the stomach and intestine, cirrhosis of the kidneys, extensive haemorrhages in mucosa of rectum, subpleural haemorrhages in left thoracic cavity. *Histology.*—Liver: Pigmentation, degeneration, and cirrhosis. Bloed and spleen smears.—Negative.
Dog 1082 (16 months old) (mixed breed)	5 gm. of ground-up plant mixed in food daily	18 days	Approximately 20 gm....	—	On the 13th day of the experiment diarrhoea appeared and a certain degree of inappetence and listlessness was present, loss in condition was noticeable and feeding of the plant was discontinued on the 18th day of the experiment in order to study the further course of the disease. Marked improvement in the condition of the animal had occurred three weeks after discontinuation of feeding of the plant and the animal appeared quite normal within a further six weeks.

Rabbits.—All four rabbits drenched with *Senecio isatideus* DC. died after having exhibited the following symptoms: Pronounced cachexia (except rabbit O, which died on the third day of dosing), progressive inappetence, anaemia and apathy, laboured respiration, and weak and accelerated heart beat.

Postmortem appearances.—Emaciation (except rabbit O) hydroperitoneum, hydrothorax, pronounced hyperaemia and slight oedema of the lungs, extensive haemorrhage into the mesentery and subserosal tissues of the big intestine; ulceration of the gastric mucosa; dilatation of both heart ventricles; atrophic or hypertrophic cirrhosis of the liver with no pigmentation; hyperaemia of kidneys and spleen.

Histology.—Liver: hyperaemia, multiple necrotic areas, cirrhosis.

Dogs.—Both the dogs that had been drenched and fed and those that had been fed only died from *Senecio isatideus* DC. poisoning. There is, however, some doubt in the case of dog 1041, which died from uraemia caused by severe disease of the kidneys. Whether the kidney lesions described were caused by the plant or not, is impossible to say.

The following symptoms were developed by dogs 1050, 1046, 1048, and 1049: Progressive cachexia, inappetence and listlessness, anaemia, diarrhoea, yawning, constipation, retching, vomiting, allotriophagia, general icterus (not seen in dogs 1050 and 1046), weak and accelerated pulse: death (dogs 1046, 1048, and 1049) was preceded by coma.

Post-mortem appearances.—(Dogs 1046, 1048, and 104): Extreme cachexia intense general icterus (absent in dog 1046), pronounced hydroperitoneum, hyperaemia of the lungs, pronounced pigmentation (absent in dog 1046), degeneration and cirrhosis of the liver, extensive ulceration of and haemorrhages in the mucosa of the pyloric portion of the stomach: subacute catarrhal enteritis, straw and grit in gastro-intestinal tract, the stomach and intestine contained a dark reddish-brown mucous substance, which proved to be changed blood.

Histology.—Liver: Cirrhotic and degenerative changes are characteristic of chronic *Senecio isatideus* DC poisoning in dogs.

From the above table it appears that rabbits, although susceptible to *Senecio isatideus* DC. poisoning, require much larger quantities of the plant to cause death than do dogs.

There is a marked resemblance in the symptoms and post-mortem lesions in dogs poisoned by *Senecio ilicifolius* DC. and in those poisoned by *Senecio isatideus* DC. As in *Senecio ilicifolius* DC. poisoning it was found that small amounts of *Senecio isatideus* DC. administered over prolonged periods did not produce general icterus, which invariably occurred in dogs taking larger amounts over shorter periods.

Dogs poisoned with *Senecio isatideus* DC. are liable to recover provided the administration of the plant is discontinued before the liver has been damaged beyond repair.

History.—Steyn (1931) proved *Senecio isatideus* DC. toxic to sheep and a horse. The latter animal exhibited symptoms which were indistinguishable from those of natural cases of "Dunsiekte" in horses.

(*f*) ***Senecio laevigatus*** **Thunb.**

(*g*) ***Senecio rigidus*** **L.**

(*h*) ***Senecio rosmarinifolius*** **L. f.**

Muir (1928) and also the author have collected these three species of *Senecio* on wheatlands in the Riversdale district. What is more they were present on lands on farms where cases of suspected bread poisoning occurred.

Marloth (1917), records *Senecio rigidus* L. under the common name of "poisonous ragwort."

Watt and Brandwyk (1932) state that they have proved *Senecio rigidus* L. poisonous to a rabbit. From experiments conducted at Onderstepoort on a large number of rabbits it would appear that rabbits are the animals least suitable for use in the determination of the toxicity of *Senecio spp.*

There is a greater possibility of *Senecio laevigatus* Thunb. and *Senecio rosmarinifolius* L. f. finding their way into threshed wheat than there is of *Senecio rigidus* L. doing so, as the latter plant is very tall and is almost exclusively found growing in very moist patches of the lands.

CRUCIFERAE.

Raphanus raphanistrum L.

Registered number.—Onderstepoort Spec. No. 6628R, 9/5/32.

Nat. Herb. No. 14274.

Common and vernacular names.—Knopherik, ramenas, jointed or white charlock, wild radish, field wall-flower.

Origin.—Sorted from a bag of wheat obtained from the Langkloof Roller Mills, Joubertina.

Parts of plant tested.—Ripe seed (+capsule).

Rabbits were drenched as follows :—

Rabbit A.—Received 105 grams of the above seed in the course of twenty-two days at the rate of 5 grams daily (except Sundays).

Rabbit B.—Received 225 grams of the above seed in the course of seventeen days at the rate of 15 grams daily (except Sundays).

Rabbit C.—Received 360 grams of the above seed, moistened twelve hours before dosing, in the course of twenty-seven days at the rate of 15 grams daily (except Sundays).

Rabbit D.—Received 310 grams of the above seed, moistened twelve hours before dosing in the course of thirty-six days at the rate of 10 grams daily (except Sundays).

Result.—Rabbit A died on the twenty-seventh day, Rabbit B on the seventeenth day, Rabbit C on the twenty-seventh day, and Rabbit D on the thirty-sixth day of the experiment after having exhibited inappetence, apathy and diarrhoea with gradual loss in condition.

Post-mortem appearances.—Hyperaemia and slight oedema on the lungs, pronounced subacute catarrhal gastro-enteritis and, in one case, subserosal haemorrhages in the peritoneal cavity.

Histology.—No specific lesions were found in the liver, kidneys, and heart.

History.—The seed of *Raphanus raphanistrum* L. contains small amounts of an irritant substance. The seed is used in tympanites, rheumatism and sciatica (Dragendorf, 1898).

Kobert (1906 writes that according to Sjollema *Brassica napus* L. and *Brassica rapa* L. contains more than one glucoside, which all develop mustard oil. These mustard oils are responsible for the production of chronic enteritis with tympanites, haemorrhagic diarrhoea, colic, stimulation of the brain, and abortus when horses and cattle ingest rape cakes over prolonged periods. Haematuria and the accumulation of haemorrhagic fluid in the thorax and peritoneal cavity were also seen. Kobert furthermore mentions that probably similar glucosides are contained in *Raphanus raphanistrum* L. which is known to have caused poisoning in animals.

Long (1917) writes: " Wild radish (*Raphanus raphanistrum* L.). As in the case of charlock, the seeds of wild radish are very acrid, and susceptible of introducing intestinal troubles if eaten by animals when mixed with cereals."

Fröhner (1919) states that *Raphanus raphanistrum* L. is one of the plants which, owing to its containing mustard oil or similar substances, will cause poisoning when ingested in large amounts.

Elaine (1922) described poisoning in horses with grain, which contained a third or a fourth part of the seeds of the wild radish. The horses showed colic and inappetence and recovered after treatment.

Wehmer (1929) writes that the seed of *Raphanus raphanistrum* L. contains 30 to 40 per cent. of fatty oil and no sinigrin, but a sinalbin-like sulphur-containing glucoside and myrosin.

Petri (1930) states that mustard oils are excreted by the lungs and kidneys and that they are severe irritants to the mucous membranes, and skin. Haematuria may be caused after the ingestion of mustard and rape cakes. The liver of animals poisoned with mustard oil are yellowish grey and soft in consistence, and microscopically show necrosis of the parenchyma. Petri mentions that Carlau saw disintegration of the liver and haemorrhages in guinea pigs and rabbits which had succumbed to mustard oil poisoning.

E. EUPHORBIACEAE.

(*a*) *Euphorbia helioscopia* L.

Registered number.—Onderstepoort Specimen No. 5179 ; 9/10/30.
Nat. Herb. No. 14267.

Vernacular names.—Melkgras ; melkbos ; wolfsmelk : milkweed ; spurge.

Origin.—Cultivated lands, vicinity of Capetown.

Habitat.—Cultivated lands southern and western Cape Province.

Parts of plant tested.—Whole plant ; fairly fresh and tested in the preflowering, flowering, and seeding stages.

It was suspected of having caused poisoning in stock running on cultivated lands. Three sheep received respectively per stomach tube 2,000 grams, 2,100 grams, and 1,750 grams of the plant in the different stages of development in the course of a few days without suffering any ill-effects (Steyn, 1931 and 1932).

History.—The plant itself as well as the " bark " was used as a purgative under the name of Herba et Cortex Esulae vel Tithymali. Latterly the latex, which is slightly irritant, was used in the treatment of syphilis (Rosenthal, 1862). Dragendorff (1898) states that this plant is poisonous and Kobert (1906) that it causes poisoning in children.

Pammell (1911) writes that Lehmann lists "*Euphorbia helioscopia*" as a poisonous plant.

According to Long (1917) sun spurge (*Euphorbia helioscopia* L.) has caused fatal poisoning to a boy who ate it "and in Germany cows were poisoned through pasturing in stubble in which the plant was growing, but there were no deaths."

Fröhner (1919) states that the following species of *Euphorbia* are toxic to stock: "*E. cyparissias*," "*E. peplus*," "*E. helioscopia*," "*E. marginata*," and "*E. lathyris*," and that their toxic properties are due to euphorbinacidanhydride. The milk of goats which has partaken of *Euphorbia helioscopia* L. is stated to have caused poisoning in human beings. Bernhard-Smith (1923) mentions euphorbin as the toxic principle of *E. helioscopia* L. and E. peplus L.

E. helioscopia L. has been reported to cause constipation and narcosis in stock (Onderstepoort File 144/512, 14/9/28).

Dunning (Onderstepoort File 144/250, 10/9/31) writes that in a feeding experiment a sheep ingested 161 ounces of this plant in the young seeding stage without developing any symptoms of poisoning.

(*b*) *Euphorbia peplus* L.

Vernacular names.—Wolfsmelk, spurge.

Habitat.—Cultivated lands southern and western Cape Province.

History.—In the early centuries this plant under the name of Herba Esulae rotundifoliae was used as a remedy for dropsy (Rosenthal, 1862). According to Kobert (1906) it causes blisters on the skin and inflamation of the mouth and intestine and according to Pammel (1911) Lehmann records it as a poisonous plant. Long (1917) and Fröhner (1919) also refer to the toxicity of this plant.

Seddon (1929) produced salivation and blood stained faeces, but not death, in a calf drenched with a watery extract of 4 ℔. of the fresh green plant in the early flowering stage.

Symptoms of Euphorbia poisoning.—On the skin the latex causes itching, redness, pimples, and in bad cases gangrene. The seeds cause symptoms of severe gastro-intestinal irritation, namely, inappetence, salivation, nausea, constipation, vomiting, pronounced diarrhoea, which may be haemorrhagic, colic, tympanites, palpitation of the heart, apath, dizziness, convulsions, unconsciousness, collapse and death.

Post-mortem appearances.—Severe acute catarrhal or haemorrhagic gastroenteritis with ulceration of the mucosa.

(*c*) *Ricinus communis* L.

Common names.—Kasterolie boom, castor oil tree.

Habitat.—A native of Southern Asia; ubiquitous; on cultivated lands.

As the castor oil plant is of frequent occurrence on cultivated lands there is a possibility of its seed contaminating mealies, beans, etc., especially those foodstuffs grown by natives and irresponsible Europeans. The danger is greatest when the harvested crops are stacked and threshed on lands where castor oil plants grow, as the possibility of contamination is greater than when the crops are threshed outside such infested lands.

It has frequently occurred that maize stored in the same hold of the ship as castor oil seed has become mixed with the latter with the result that serious mortaility has occurred in cattle and horses (Legal Notes, 1931 and 1932).

With regard to cakes and meals as feedingstuffs for stock a content of 0·02 per cent. seed is regarded as dangerous.

History.—Rosenthal (1862) states that *Ricinus communis* L. occurs in many varieties of which one of the most wellknown ones is *Ricinus africanus* Willd. Castor or ricinus oil is one of the most extensively used mild oily purgatives.

In 1864 Tuson mentioned that the seeds of *Ricinus communis* L. contains ricinin and described a method of isolating it (Husemann, 1882).

Castor seeds also contain the toxalbumin ricin; the root of this plant is used in kidney troubles and the leaf in abscesses (Dragendorff, 1898).

The seeds contain colouring matter and a large amount of oil, proteins, enzymes, and ricin (Kobert, 1906).

Long (1917) writes that "according to Cornevin four seeds suffice to cause accidents in man, eight lead to very grave results and beyond that number death may ensue." He also mentions that the seeds have been found as an impurity in linseed cake and maize meal.

Pammel (1911), Long (1917). Fröhner (1919), Byam and Archibald (1921), Thomson and Sifton (1922), Lander (1926), and Petri (1930) all refer to the toxicity of the castor oil plant.

Heffter (1924) refers extensively to the methods of isolating ricin from castor seeds and to its actions in vitro and in vivo.

Cases of asthma resulting from the inhalation of castor bean dust in a castor oil factory have been described by Figley and Elrod (1928).

Ratner and Gruehl (1927–1928) produced anaphylaxis in guinea pigs by allowing them to inhale castor bean dust and injecting them intravenously with an extract of this dust after an incubation period of three weeks. Some guinea pigs died from ricin poisoning caused by the inhalation of castor bean dust and showed a severe haemorrhagic condition of the lungs.

Dodd (1932) made a valuable contribution to the study of the presence of castor seed in feedingstuffs. Dodd realises that the determination of the percentage of castor seed in feedingstuffs is not of great value as in practically all cases the castor seed is unevenly distributed. As new contracts with regard to feedingstuffs specify limits of contamination with castor seed, analysts are, however, forced to state percentages. With regard to the possibility and probability of the various castor seeds varying in toxicity Dodd rightly remarks that all castor seeds should be regarded as deadly. He describes the method employed by him in the detection and identification of castor seeds in cakes and states that seeds which might easily be mistaken for castor include grape or raisin seed, ucuhuba seed, croton seed and curcas seed. The two former seeds are harmless whilst the two latter are more poisonous than castor seed. He says: "It is usual to return castor, croton or curcas as castor seed." The percentage of castor seed present in the cakes is calculated by multiplying the weight of the husk found by 5.

Symptoms of poisoning.—According to the above-mentioned authors and Bornemann (1922) and Mariott (1922) the following are the symptoms of castor bean poisoning in human beings and animals.

(1) *Human beings.*—The symptoms are those of very severe gastro-intestinal irritation, namely burning pains in the throat and stomach, salivation, nausea, vomiting, colic, diarrhoea, thirst, small rapid pulse and also cramps of the calves and abdominal muscles, drowsiness, measle-like eczemata, cyanosis, and icterus.

Post-mortem appearances.—Acute gastro-enteritis sometimes accompanied by erosions in the stomach.

(2) *Animals.*—Haemorrhagic interitis with its accompanying symptoms, staggering, dullness of vision, marked apathy, heart weakness, paralysis, somnolence, convulsions, muscular spasms, fever, shivering, coma and death. In some cases toxic laminitis was noticed. Death occurs within one to three days in acute poisoning.

Post-mortem appearances.—Acute gastro-enteritis; haemorrhages in the cortex of the kidneys and subpleural tissues of the lungs; degeneration of the myocard; subendocardial and subepicardial haemorrhages; haemorrhages in the serosa of the body cavities and in the organs; haemorrhagic ascites.

Histology.—Fatty degeneration of the myocard, congestion haemorrhages and accumulation of fat in the liver and irregular areas of parenchymatous disintegration; chronic poisoning causes cirrhosis of the liver; spleen shows increased pulp but owing to the disappearance of the lymphocytes the follicles are few and small, the haemorrhages and areas of disintegration in the spleen are due to occlusion of the capillaries by fibrin; the bonemarrow is very soft and mottled due to hyperaemia and haemorrhages; the marrow appears to produce abnormal blood elements; the circulating blood shows eosinophile leucocytosis and polychromasia (due to damaged blood forming apparatus); the kidneys, which excrete ricin, show fatty degeneration and necrosis of the tubuli epithelium.

Toxic principle.—The active principle of castor oil seed is the toxalbumin ricin, which is a very severe irritant and is much more poisonous when administered parenterally, than when taken per os. By reason of its proteid character ricin when injected subcutaneously produced an immunity to castor bean poisoning.

Toxicity of castor seeds.—Miessner (Fröhner, 1919) gives the following quantities as the lethal doses of castor bean seed administered in one dose:—

Horses................	0·1	gram per Kg. body weight.
Cattle................	2·0	,, ,,
Sheep................	1·25	,, ,,
Goats................	5·5	,, ,,
Pigs................	1·4	,, ,,
Rabbits................	1·0	,, ,,
Geese................	0·5	,, ,,
Fowls................	14·0	,, ,,

If the feeding of small quantities of castor bean seed is continued it has a cumulative action.

The seed is much more poisonous when administered subcutaneously.

At Onderstepoort the fresh immature seeds were found to be highly toxic to rabbits.

Treatment.—Symptomatic treatment must be applied as no specific antidote is known.

Detection of castor bean in foodstuffs.—This can be done botanically and biologically by the precipitation and conglutination tests.

F. GRAMINEAE.

(*a*) *Lolium temulentum* L. var. *macrochaeton* A. Br.

Registered No.—Onderstepoort Spec. No. 6628 Q; 22/3/32. Nat. Herb. No. 14265.

(*b*) *Lolium temulentum* L. *var. muticum* Boiss.

Registered No.—Onderstepoort Spec. No. 6574; 22/3/32. Nat. Herb. No. 14266.

Common names.—Drabok, darnel, cheat, " Tares " of the Bible. German: Taumellolch, Tobkrout.

Habitat.—Occurs extensively in cultivated lands especially in the southern and western Cape Province.

Origin.—Spec. 6628 Q (N.H. No. 14265) sorted from a bag of wheat contaminated with weed seeds obtained from the Langkloof Roller Mills, Joubertina.

Specimen 6574 (N.H. No. 14266).—Sorted from a consignment of wheat contaminated with weed seeds obtained from the Roller Mills, Riversdale.

Specimen 6628*Q.*—These darnel seeds were undersized and discoloured and had a mouldy smell. The degree of fungus infection was 100 per cent. When chewed they at first had a slight sweetish starchy taste, which after a few minutes became bitter.

Dr. A. C. Leemann of the Division of Plant Industry examined this specimen of drabok mycologically. The results of his investigation are incorporated in an article published elsewhere in this report.

Two rabbits ingested 3,370 grams and 3,225 grams of these fungus-infected darnel " seeds " in the course of thirty-eight days respectively. No additional food was given to these rabbits during the period of experimentation.

Result.—Not only did these " seeds " prove harmless but they provided also in all the necessary nutritive substances essential for the maintenance of health and growth of the experimental rabbits.

Pig 789 ingested 4,000 grams of this fungus-infected drabok in three days without suffering any ill-effects. No additional food, except a small quantity of milk mixed with the drabok meal was given.

Dog (no number) took 1,250 grams of this drabok meal in the course of ten days without any detrimental effects. The raw meal was made into porridge with a small quantity of milk.

The above experiments would have been conducted over longer periods had more drabok been available.

Specimen 6574.—These darnel " seeds " were of normal size and appeared healthy. Three rabbits ingested in the course of seventy days 5,585 grams, 4,655 grams, and 6,065 grams of the " seeds " respectively. No additional food was given.

Result.—These seeds also proved harmless and provided all the food requirements essential for the maintenance of health and growth of the experimental animals.

In addition to the above experiments with darnel a number of other feeding experiments with the seed have been conducted on rabbits and horses at Onderstepoort in the course of the last five years, with negative results.

History.—For centuries this plant has been regarded as harmful to health. It is held to be the "tares" mentioned in the Bible which were sown amongst the enemy's wheat.

Huseman and his co-publisher (1882) mentions that an impure bitter substance, named loliin, had been isolated from the seed of *Lolium temulentum* L.

Dragendorff (1898) states the seeds to be poisonous and that the meal was used as an analgesic and in the treatment of skin diseases. The seeds contain temulentin, loliin and temulentic acid and according to Hofmeister temulin.

Kobert (1906) correctly remarks that many of the cases of poisoning in Russia, Germany, France, etc., ascribed to darnel could have been caused by other extraneous material contained in the wheat.

The cases of darnel poisoning referred to by Willmot and Silberbauer (1931) will be dealt with under "Discussion."

For further references see Leemann's article: "A short summary of our botanical knowledge of *Lolium temulentum* L." published elsewhere in this report.

Symptoms of poisoning.—[Kobert (1906); Pammel (1911); Long (1917); Fröhner (1919); Byam and Archibald (1921); Thomson and Sifton (1922); Lauder (1926)].

(*a*) *Human beings.*—Pronounced apathy; sleepiness; staggering; giddiness; trembling; mydriasis; a feeling of pressure in the epigastrium; nausea; vomiting; and later, painful cramps of the stomach; diarrhoea; heartweakness.

Barger (1931) (p. 29) writes: "In Germany and elsewhere the darnel ("*Lolium temulentum,*" zizania, the tares of Scripture ?) was considered by some to be the cause of the Kriebelkrankheit and more plausibly, since this grass does indeed contain a narcotic poison. Hussa observed a number of cases of actual poisoning by rye containing 16 to 22 per cent. of darnel seeds; the symptoms were frontal headache, giddiness, rumbling in the ears, gastric pains, twitching of the tongue, difficulty in swallowing and in speech, vomiting, diarrhoea, fatigue, cold sweat, and trembling of the limbs. The patients declared that they felt completely intoxicated. There is here a slight resemblance to some of the symptoms of ergotism, but various observers agree that the effects of "Lolium" poisoning are of short duration; after a sound sleep Hussa's patients were practically normal next day."

Post-mortem appearances.—Darnel very rarely or never causes death in human beings. No specific lesions are described in the available literature.

(*b*) *Animals.*—Mydriasis; vertigo; uncertain gait; trembling; laboured respiration; slow and small pulse; convulsive movements of the head and limbs; paralysis (in pigs); unconsciousness; colic; spasms.

Post-mortem appearances.—As a rule the post-mortem is negative; rarely slight gastro-enteritis; hyperaemia of the lungs and hyperaemia of the brain and spinal cord are seen.

Toxic principle.—It is at present generally held that darnel is poisonous only when infected with fungi. According to Wehmer (1929) the active principle of darnel is the alkaloid temulin.

This plant is referred to in the article "Fungi in Relation to Health in Man and Animal" published elsewhere in this report.

Toxicity of darnel.—Cornevin's (Lander, 1926) lethal doses are :—

Horse....................	7 grams per Kg. body weight.
Dog......................	18 „ „

Ruminants and birds are supposed to be less susceptible.

Pammel (1911), quoting Hackel, says that the toxicity of darnel is due to a narcotic principle, loliin, which causes trembling, eruptions, and confusion of sight in man and flesh-eating animals, and particularly in rabbits, but it has no effect on swine, horned cattle or ducks.

Treatment.—Symptomatic.

G. LEGUMINOSAE.

Vicia sativa L.

Registered number.—Onderstepoort Spec. No. MN ; 18/4/32. Nat. Herb. No. 14271.

Vernacular names.—Common vetch ; wilde wieke ; "tares."

Origin.—Seed collected on the suspected wheatland at Modderfontein, Humansdorp, and sown at Onderstepoort. Experiments were conducted with the seed collected at Onderstepoort.

On moistening the ground up seed with water a typical bitter almond smell was emitted, and the picrate paper test for hydrocyanic acid was strongly positive. Dr. C. Rimington, Onderstepoort, found these seeds to contain 64·8 mgm. of hydrocyanic acid per 100 grams of seed.

A rabbit drenched with 20 grams of these seeds developed typical symptoms of hydrocyanic acid poisoning within five minutes and died within one and a quarter hours of dosage.

The post-mortem appearances were those of hydrocyanic acid poisoning. A large amount of this acid was detectable in the stomach contents by the picrate paper test.

History.—*Vicia sativa* L. is cultivated as a fodder plant especially for the feeding of cattle. Husemann and his co-publishers (1882) described methods of isolation of vicin and convicin from *Vicia sativa* L. peas. When vicin is heated in dilute sulphuric acid divicin sulphate crystalises out on cooling.

Dragendorff (1898) mentions that among other things cholin and betain are contained in the seed of this plant.

Kobert (1906) writes that *Vicia sativa* L. has caused poisoning in horses, cattle, and pigs with symptoms similar to those of lupinosis. Horses fed with this plant developed pronounced icterus and at post-mortem there was enlargement of the liver, which was of an orange yellow colour. In other cases horses showed loss in condition, alopecia and icterus ; the postmortem lesions were enteritis, brownish discolouration and swelling of the liver. Similar symptoms and post-mortem lesions were caused in pigs, which had partaken of this plant.

Pammel (1911) states that this is another weed commonly found in wheat screenings, and that it is harmful to pigs but not to cows. Fröhner (1919), quoting Wenke and Mason, describes weakness, paralysis of hindquarters, blindness, laminitis, trismus and death in horses poisoned with *Vicia sativa* L.

Anderson, Howard, and Simonson (1925) investigated the toxicity of *Lathyrus sativus* L. and state their experiments to indicate that *Lathyrus sativus* L. itself is harmless and that the toxicity ascribed to it is due to contamination with the seeds of *Vicia sativa* L. They, furthermore, state that the seeds of the latter plant contain a glucoside, vicin, and a cyanogenetic glucoside, vicianin, which is closely related to amygdalin. Vicin on hydrolysis yields a base divicine and d-glucose.

The authors were able to kill guinea pigs by injecting them subcutaneously with 0·6 mgm. of divicine per Kg. body weight. Violent and continuous clonic convulsions of the whole body were exhibited for about an hour and then progressive paralysis set in until death supervened within some hours of injection. Intense congestion of and about the brain and spinal cord was found at post-mortem.

Owing to the small amount of vicin and vicianin they were unable to determine whether these substances are toxic or not.

Anderson and his collaborators produced poisoning in ducks and monkeys by feeding them on a diet containing a high percentage (50 per cent.) of *Vicia sativa* L. seeds.

The ducks exhibited ataxy, walking in circles, convulsions, paresis and a kind of writhing contortion of the whole body with extreme retraction of the head and neck, and died from the thirteenth to the hundred and twenty-fifth days of the experiment.

Post-mortem lesions.—Oedema of the subcutaneous tissues of the head and haemorrhages over the surface of the skull; superficial congestion of the brain and hyperaemia of the cerebellum, medulla and upper cord.

In the monekys the symptoms appeared from the sixth to the five-hundredth day of the experiment. The animals became less active, crouched in the cages, were unable to sit up and were constantly grinding the teeth. They showed fibrillary twitchings of the muscles of the arms, legs and flanks and violent convulsions of the whole body lasting from five to ten minutes. They yawned frequently, were hyperexcitable and showed symptoms of paralysis. They, furthermore, state that vicin itself is apparently non-toxic, but that during digestion in the stomach it is hydrolysed into divicin, which they proved poisonous.

Wehmer (1929) states that the seeds of *Vicia sativa* L. contain vicin, convicin, and that they yield prussic acid and benzaldehyde.

Wernery (1929) mentions that the seeds of different species of Vicia contain vicianin, which through enzyme-action liberate prussic acid.

Deaths in mules and horses running on harvested lands in the Western Cape Province have been ascribed to this plant. These animals showed a stiff gait and became paralysed, particularly in the fore-quarters. Patchy inflammation of the intestine was the only lesion seen at post-mortem. (Onderstepoort File 144/3234, 5/12/31).

A number of pigs which had been fed "Chilean peas," which consisted mainly of the common vetch (*Vicia sativa* L.) became ill and died suddenly.

The post-mortem revealed gastritis and patchy enteritis. On incubating these peas with water 0·018 per cent. prussic acid was found in them and this apparently was the cause of death (Clough, 1931).

Stockman (1931) isolated a "poisonous acid" from the seeds of the common vetch. By injecting this acid subcutaneously he produced marked general weakness and paralysis on monkeys, rabbits, and frogs.

H. POLYGONACEAE.

Rumex acetosella L.

Vernacular names.—Boksuring, steenboksuring, dock, Sheepsorrel.

Habitat.—Cultivated lands. Very prevalent in southern and western Cape Province.

The "seeds" of this plant were found in specimens of wheat received from the southern and western Cape Province.

History.—Rosenthal (1862) and Dragendorff (1898) mention that the root and seed of this plant are used as toxic-astringents in diarrhoea, and the leaves, which contain potassium oxalate as an antiseptic and remedy in scurvy.

According to Kobert (1906) *Rumex acetosella* L. contains acid potassium oxalate.

Long states that children have been poisoned by eating large amounts of *Rumex acetosa* L.

Fröhner (1919) reports poisoning in sheep grazing on harvested lands heavily overgrown with *Rumex acetosella* L.

Graig and Kehoe (1921) fed 147 ℔. of *Rumex acetosa* L. for a month to a bull with negative results.

Wehmer (1929) states that *Rumex acetosella* L. contains potassium oxalate.

Symptoms of poisoning.—The symptoms resemble those of subacute or chronic oxalic acid poisoning.

Animals.—Drunkenness, swaying gait, salivation, muscular tremors, dilatation of the pupils, feeble, slow and intermittent pulse, convulsive contraction of the lips, accelerated and stertorous breathing, tetanic contraction of the muscles of the neck, back, and limbs, profuse sweating, inappetence, diarrhoea, apathy, symptoms of paralysis, death occurs in convulsions.

The milk of affected cows is said to be made into butter with difficulty.

Post-mortem appearances.—Acute catarrhal gastro-enteritis with haemorrhages on the gastro-intestinal mucosa.

Treatment.—Limewater, calcium carbonate, diuretics, furthermore symptomatic treatment.

Oxalic acid and oxalate poisoning are fully described by Witthaus (1911), Petri (1930), and Glaister (1931).

I. SOLANACEAE.

(*a*) *Datura stramonium* L.

Vernacular names.—Stinkblaar, olieblaar, olieboom, thorn apple, Pietjie Laporte, Jimson weed.

Habitat.—Very common on cultivated and waste lands and along river.

At Onderstepoort a rabbit received 100 grams of the ripe seed on one day and a sheep 1,000 grams of the ripe seed administered at the rate of 500 grams daily without developing any symptoms of poisoning (Steyn, 1931).

History.—*Datura stramonium* L. was used in witchcraft practice in olden times and was later extensively used in homocidal poisoning (Lewin, 1920).

Rosenthal (1862) states that the leaves and seed contain a most poisonous narcotic alkaloid, daturin. This plant is used as a remedy in cases of neuralgia, spasms, epilepsy, stomach cramps, chronic rheumatism and is smoked to relieve asthma.

Hutcheon (1903) writes : " The seeds and young growing plants of *Datura stramonium* or Stinkblaar, as it is called, are very poisonous to young ostriches ; I have seen them die off very rapidly from eating the leaves of the young plants.

Veterinary Surgeon Sinclair reports (*vide Agric. Jour.*, Vol. 13, p. 550) equally serious cases resulting from ostrich chicks eating the seeds obtained from the fruit of the previous season."

No marked lesions were present as the poison acts on the central nervous system causing a dull, sleepyc ondition terminating in complete collapse. Some birds showed delirious excitement and staggered about before the comatose condition set in. The treatment recommended is castor oil and strong coffee.

Kobert (1906) mentions that the active principle of the thorn apple is atropine.

According to Bryant (1909) the leaves of this plant are freed from the midribs and then laid over painful wounds and sores.

According to Pammel (1911) the seeds are the most poisonous part of the plant.

South African specimens of " *Datura stramonium* " examined at the Imperial Institute contained 0·49 per cent. of total alkaloid, the chief alkaloidal constituent being hyoscyamine (Editorial, 1916).

Mitchell (1923) records a case of Datura poisoning in human being at Vrede, Orange Free State.

Watt and Brandwyk (1927) recorded poisoning in mine boys in South-West Africa through eating beans contaminated with seeds of *Datura stramonium* L.

Beyers (1930) reported cases of poisoning in natives in the Somerset East district due to the eating of " boermeal " admixed with seeds of *Datura stramonium* L.

Symptoms of poisoning.—(Kobert, 1906 ; Witthaus, 1911 ; Long, 1917 ; Fröhner, 1919 ; Byam and Archibald, 1921 ; Thomson and Sifton, 1922 ; Watt and Brandwyk, 1927 ; Gimlette, 1929 ; Beyers, 1930 ; Petri, 1930 ; and Glaister, 1931.)

(1) *Human beings.*—The symptoms vary according to the size of the dose and the age of the victim and may appear within a few minutes after ingestion of the seeds. The following symptoms may be exhibited : Giddiness, yawning, dryness of the throat and thirst, attempts to swallow provoke spasms of the pharynx and may resemble hydrophobia to a certain extent, bitter taste and burning sensation in the mouth ; impairment of vision, power of standing is lost and on attempting to walk patient staggers as if intoxicated, progressive restlessness, which may develop into wild delirium, pupils widely dilated, difficult and incoherent speech, affected people try to climb walls and trees, pull on imaginary ropes, children run about naked and on all fours, pick at imaginary objects, and search the bedding most vigorously for some lost article, " tries to thread imaginary threads and tries to pick them from the tips of his fingers or he constantly gazes at his fingers and keeps passing his thumb over them in a most peculiar way ". (Gimlette, 1929), laughing is common, and there is a tendency to discard clothes, when trying to read " the letters run over the pages

like so many ants," flushed (scarlatinal) face and a scarlatinal rash with itching may appear upon the skin of the body and extremities, the pulse is at first hard and full and very much accelerated (up to 200 per minute) and in the course of time becomes intermittent, irregular and ultimately imperceptible, the skin may be hot or cold and dry, vomiting is uncommon, all reflexes are exaggerated, delirium passes into exhaustion, coma supervenes and terminates in death. Watt and Brandwyk (1927) describes vomiting and purging in a few of the victims.

Post-mortem appearances.—Dilatation of the pupils, hyperaemia and oedema of the lungs, hydrothorax, pronounced congestion of the meninges of the brain, with bloody serum in the ventricles, and punctiform haemorrhages in the brain substance, hyperaemia of and haemorrhages in mucosa of stomach and small intestine.

After effects of Datura poisoning.—As the toxic principles of *Datura stramonium* L. are excreted slowly the after effects may persist for quite a number of days. There may be general weakness, dilatation of the pupils, thirst, impairment of the memory and difficulty in walking.

Toxicity.—It is impossible to state definitely the toxic dose of the seed as the amount of active principle varies considerably in the plant growing in different localities.

Treatment.—Treatment of cases of atropine poisoning and chemical analysis of the gastro-intestinal contents of such victims are described by Glaister (1931) and Byam and Archibald (1921).

(2) *Animals.*—Cattle and horses are considered equally susceptible, whilst they are held to be less susceptible than carnivora.

The symptoms in ostriches have already been referred to (Hutcheon, 1903).

Other animals show dryness of the tongue and buccal mucous membrane, dilated pupils, an accelerated, irregular and weak pulse, excitement followed by paralysis, tympanites, staggering and death from asphyxia and heart failure.

Post-mortem appearances and histology.—The appearances in acute cases are essentially those of asphyxia.

In chronic cases of atropine poisoning wheals, blisters, petechiae and scarlatina-like eczemata appear on the skin. Anima and Metzner found enlarged alveoli and also diminished alveoli with shrivelling of the ducts in the salivary glands of animals poisoned with atropine. The blood picture appears normal in atropine poisoning (Petri, 1930). Agapi (Petri, 1930) saw extensive haemorrhages in the parenchym of lungs of mice repeatedly injected with atropine.

Toxic principle.—In all parts of the plant hyoscyamine is the chief alkaloid and a small amount of atropine and scopolamine is also present (Wehmer, 1929). It is quite possible that the ratio of the percentages of these three active principles in the seed of this plant growing in different localities may vary as this is known definitely to occur in other plants with more than one active principle (for example, *Digitalis* glucosides).

Differential diagnosis.—Clinically and pathologic-anatomically meat and fish poisoning resemble atropine poisoning. In the case of suspected Datur poisoning, especially in natives and coloured people, methyl alcohol poisoning is differential diagnostically of great importance.

(b) *Datura Tatula* L.

Common name.—Purple thorn apple, purple stramonium, blou stinkblaar, blou olieboom.

Habitat.—As in *Datura stramonium* L.

Two sheep received per stomach tube 750 grams of fresh leaves of the plant in the late flowering stage on each of two consecutive days and 500 grams of the ripe seeds on each of two consecutive days respectively.

Result.—Negative (Steyn, 1929).

History.—Pammel (1911), referring to the toxicity of this plant remarks that the seeds are especially poisonous.

Thomson and Sifton (1922) state that this plant has similar effects to those of *Datura stramonium* L.

According to Bernhard-Smith (1923) the active constituents of *Datura tatula* L. are atropine, hyoscyamine, and hyoscine.

Beyers (1930) referring to *Datura stramonium* L. poisoning writes: "In conclusion it may be of interest to state that about fifteen years ago I attended a few small children who were similarly affected after sucking the honey from the nectar of the large purple flowers of the stinkblaar."

III. LEGAL ASPECT.

Regulation 12 (7) of the Food, Drugs, and Disinfectants Act, No. 13 of 1929, states: "Every mill in which grain is milled for human consumption shall be provided with efficient sieving and winnowing appliances so as completely to remove the seeds of *Senecio* (Sprinkaanbos) and any other poisonous or unwholesome seeds or matter. Any person selling any flour or meal containing such seeds or matter shall be guilty of an offence."

As all the evidence, both circumstantial and experimental, gathered in the past and present investigations, points very strongly to bread poisoning being due to the presence of portions of Senecio plants in the wheat used for household purposes, and as no evidence whatsoever has as yet been brought forward to disprove this contention, it would seem advisable to introduce legislation whereby all growers of foodstuffs for man and animal will be forced to keep the lands free from all species of *Senecio*. The eradication of *Senecio* from cultivated lands will not entail an unreasonable amount of work or expense as these plants are large and very easily uprooted.

The author has seen fallow lands overgrown with *Senecio ilicifolius* Thunb. and no attempt was made to eradicate this weed. It is due to such irresponsible persons that weeds are allowed to spread instead of being eradicated.

It is a custom among many wheatgrowers to market their best and clean wheat and to use for household purposes that which is contaminated with weeds. The enforcement of the above Regulation 12 (7) would be difficult in these cases. Under these circumstances a much safer procedure would appear to be the proclamation of those species of Senecio growing on cultivated lands as noxious weeds.

IV. DISCUSSION.

At Onderstepoort seeds of the following weeds were sorted from wheat obtained from areas where cases of suspected bread poisoning occurred: *Lithospermum arvense* L. (very rare), *Silene gallica* (2 per cent.), unripe and ripe *Senecio* seed heads (very rare), *Eruca sativa* Mill. (2 per cent.), *Rephanus raphanistrum* L. (2 per cent.).*Lolium temulentum* L. (up to 33 per cent.), *Vicia sativa* L. (1·5 per cent.), *Malva parviflora* L. (0·5 per cent.), and *Rumex acetosella* L. (rare). In addition to these seeds the following substances were found: Legs and other parts of lizards, parts of beetles, bird droppings, small stones, stems of plants up to two inches long, and parts of leaves of different kinds of plants.

Furthermore, oats, barley, and lucerne seed were present in some specimens of wheat.

A. PLANTS CONCERNED IN BREAD POISONING AND IN POISONING BY OTHER FOODSTUFFS CULTIVATED ON LANDS.

(a) *Agrostemma Githago* Linn.

Thunberg (1823) recorded this plant is wheat grown near Fransch Hoek and according to Burtt-Davy it was found on cultivated land near Pretoria. The author is unaware of the presence of this plant on cultivated lands in areas where " bread poisoning " occurs. From the foregoing information it is, however, evident that this plant when growing on wheat lands constitutes a grave danger to man.

(b) *Centaurea picris* DC.

This plant was proved poisonous to sheep and as it grows on cultivated lands it is likely to find its way into foodstuffs which are grown on such infested lands and which are threshed carelessly.

(c) *Senecio burchellii* DC.

The plant obtained from the Humansdorp Commonage produced no symptoms of poisoning in rabbits, dogs, or sheep at Onderstepoort. Chase, however, proved it poisonous to horses, consequently it should be regarded as a dangerous weed on wheat lands. The plant was most probably responsible for the typical symptoms of Senecio poisoning exhibited by a horse belonging to Mrs. de Bruyn, Modderfontein, Humansdorp district. The cases of suspected bread poisoning on this farm were most probably due to *Senecio Burchellii* DC., which grows very abundantly in one corner of the wheat land. There is, however, a possibility of *Senecio ilicifolius* Thunb. also having played a role in these cases of poisoning, as a few specimens were found on the land through not acutally mixed with the wheat and it is quite likely that in some years it may be found growing amongst the wheat.

(d) *Senecio ilicifolius* Thunb.

This plant obtained from lands on a farm where " bread poisoning " occurred, caused symptoms of poisoning and post-mortem lesions in dogs very similar to those seen in cases of " bread poisoning " in human beings. Rabbits were apparently not affected by large amounts of this plant. The results of experiments upon dogs show that small amounts of the plant administered over prolonged periods do not cause clinical icterus nor was pigmentation of the liver seen at post-mortem, whilst larger quantities of this plant administered over comparative short periods invariably produced sever clinical icterus and at post-mortem intense pigmentation of the liver was present.

(*e*) *Senecio isatideus* DC.

This plant has not yet been recorded as occurring on cultivated lands in areas where bread poisoning occurs, it is however, discussed here as it possibly may spread and some day find its way on to wheatlands. The plant caused poisoning in dogs and rabbits with symptoms and post-mortem lesions closely resembling those seen in dogs poisoned with *Senecio ilicifolius* DC. The latter plant was found to be much less poisonous than *Senecio isatideus* DC. in all the experiments conducted at Onderstepoort.

Like *Senecio ilicifolius* Thunb., this plant when given in small amounts, produced no clinical icterus in dogs and no pigmented liver at post-mortem, whilst with larger amounts clinical icterus and a pigmented liver were present.

These results would tend to explain the extremely rare occurrence of icterus in suspected cases of Senecio poisoning in human beings, as it must be accepted that the victims of bread poisoning ingest very small amounts of Senecio with the bread. Dr. G. de Kock, Deputy Director of Veterinary Services, Onderstepoort, is engaged upon an investigation into the pathology of Seneciosis and an article treating with this aspect of Senecio poisoning will be published by him.

(*f*) *Raphanus raphanislrum* L.

This plant is known to be poisonous and at Onderstepoort its seed (+ seed capsules) sorted from wheat was proved to be poisonous to rabbits.

(*g*) *Euphorbia helioscopis* L.

It is recorded as toxic. Experiments with the plant received from Capetown and with material grown at Onderstepoort were negative.

(*h*) *Euphorbia peplus* Linn.

No tests have been conducted at Onderstepoort. It is, however, recorded as toxic.

(*i*) *Ricinus communis* L.

There are a number of cases on record of the seed of this plant having been found in maize and of its having caused serious poisoning in stock fed with such maize. Castor oil trees should, therefore, not be allowed to grow on or in the neighbourhood of cultivated lands.

(*j*) *Lolium temulentum* L.

All the experiments conducted at Onderstepoort on horses, pigs, and rabbits with fungus-free and fungus-infected drabok yielded negative results. Rabbits are considered to be the animals most susceptible to drabok poisoning.

The fact that large numbers of human beings, especially the coloured people, in the southern Cape Province constantly eat bread prepared from wheat very heavily contaminated with drabok without suffering any or very slight ill-effects tends to prove that drabok poisoning is of very rare occurrence or does not ocur at all.

Several people have informed the author that they are well acquainted with the symptoms of poisoning by bread containing a high percentage of drabok. These symptoms, which are more liable to occur when such bread is eaten soon after being baked and when still warm, are dizziness, headache, and sleepiness.

The cases of suspected bread poisoning which occurred at the farm Palmietfontein (Oudam), Clanwilliam district, and which were suspected by Willmot and Silberbauer to be drabok poisoning, were most probably cases of *Senecio ilicifolius* Thunb. poisoning according to the symptoms and post-mortem appearances described in the affected cases. During subsequent investigations *Senecio ilicifolius* Thunb. was found growing amongst the wheat.

It is, however, generally held by authorities in Europe that drabok is poisonous when infected with a fungus termed *Endoconidium temultentem.* The symptoms and post-mortem appearances attributed to poisoning with drabok are of such a nature that they cannot be confused with *Senecio* poisoning.

(*k*) *Vicia sativa* L.

At Onderstepoort a rabbit was killed by a small amount (20 grams) of this seed with symptoms typical of prussic acid poisoning. Chemical tests revealed the presence of a large amount of prussic acid in the form of a cyanogenetic glucoside, vicianin.

Furthermore, the plant and its seed have been proved poisonous by other investigators and the toxic principles considered to be divicine and an acid.

(*l*) *Rumex acetosella* L.

Although this plant is considered poisonous and it is recorded that children were poisoned by partaking of it, it is not likely to find its way into bread in such amounts as would be detrimental to health, except in cases of gross carelessness.

(*m*) *Datura stramonium* L.

This is a known poisonous plant. Experiments conducted at Onderstepoort with the ripe seed and the green plant on sheep and rabbits yielded negative results.

The presence of the seed has been recorded in beans and wheat (and may also find its way into maize) and has caused poisoning in human beings.

(*n*) *Datura tatula* L.

The above information is also applicable to this plant.

Attention should also be paid to *Centaurea picris* DC. in view of the fact that it has been proved poisonous to sheep.

Osteospermum moniliferu L. (bieton, boete bossie, bok berries, brother berries, bushtick berry) and *Malva parviflora* L. (mallow, kiesieblaar), should be mentioned here. The former plant was found by Muir (1928) on wheatlands and he states that it is regarded as toxic. *Malva parviflora* L. is held by many farmers to cause shivers in stock, especially in horses when they are worked soon after having ingested the plant. Dodd and Henry (1923) proved this plant as one of the causes of shivers or staggers in stock.

B. ARE SPECIES OF *Senecio* CONCERNED IN THE SO-CALLED "BREAD POISONING" IN HUMAN BEINGS?

The one and only way to prove definitely that the species of *Senecio* proved toxic to animals are also the cause of bread poisoning in human beings will be to experiment on human beings. As this method of investigation is out of the question, and as we have to rely on the results of experiments on animals, we can only state that this or that plant, whose actions on human beings are unknown, will "most probably" be poisonous to human beings as it causes poisoning in animals. The problem is more complicated by the fact that the different species of animals vary to a considerable extent in their susceptibility to poisons. Furthermore, some poisons attack different organs in the different animals (for example, *Crotalaria dura* and *Crotalaria burkeana* poisoning in horses and cattle). It is for the latter reasons that we should be cautious in drawing conclusions as to the effects on human beings of substances, which have been proved poisonous to animals.

The following facts, however, point very strongly to *Senecio spp.* being concerned in bread poisoning:--

(*a*) There is a marked similarity between the symptoms and post-mortem appearances and also in the microscopical lesions found in livers in cases of bread poisoning and those seen in animals, especially dogs, poisoned by species of *Senecio*.

(*b*) Cases of bread poisoning have only occurred in those areas where *Senecio spp.* grew on the wheatlands and usually in families belonging to the poorer classes, who paid no or very little attention to the presence of weeds in the wheat used for household purposes.

(*c*) A natural case of what was most probably Seneciosis in a horse due to the ingestion of *Senecio Burchellii* DC. was seen on a farm (Modderfontein, Humansdorp district) where seven cases of suspected bread poisoning had occurred. A corner of the land on which the wheat for household purposes was grown, was found heavily overgrown with this plant.

(*d*) Muir (1931) reported "that since measures were taken to ensure better sifting of wheat, we have had a cessation of cases of Senecio poisoning here (Riversdale area). We are hoping that this is not a coincidence, but there has been no reason to think that it is."

C. Circumstances favouring Bread Poisoning.

These are:—

(*a*) Threshing and grinding wheat in machines fitted with deficient winnowing and sieving appliances which will allow to pass through seeds and other parts of weeds. Even machines with fairly well adjusted winnowing and sieving appliances will allow to pass through weed seeds of approximately the size of wheat grains. Such weed seeds are darnel, *Agrostemma githago* L., unripe and ripe flower heads of *Senecio spp.*, etc. Small portions of the plants other than the seed may also find their way into the wheat. Specimens of wheat used for human consumption and examined at Onderstepoort were found to contain sticks up to two inches long. This is not surprising when it is considered that quite a number of wheat growers, especially those beloning to the poorer classes, thresh and grind the wheat themselves in old fashioned machines. It is quite customary amongst many wheat growers to sell the best and cleanest wheat and retain that contaminated with extraneous weeds for their own use. Furthermore, millers are instructed by some wheat growers not to adjust the above-mentioned appliances too finely as that would result in the loss of too much wheat.

(*b*) Dry years: In years of deficient rainfall the wheat does not grow to normal height and it is then reaped close to the ground with the result that there is a greater possibility of the wheat becoming contaminated with poisonous weeds.

(*c*) A grave danger is that poisonous weeds (especially *Senecio spp.*) frequently occur in patches on wheatlands with the result that during threshing some bags of wheat become heavily contaminated with these seeds, whilst the remaining bags may not contain a trace of these weeds (" pocket contamination ").

It is for this reason and also because bread poisoning is essentially a chronic disease that the collecting of specimens of wheat and meal at the time cases of bread poisoning occur, is of comparatively little value in the investigation of the prevalence of weeds in the wheat concerned.

I would like to draw attention to the fact that in the usual course of events suspected wheat samples are examined for the presence of *Senecio* seed heads, and that in some areas (or in some years in the same area) the *Senecio* plants may not have reached the flowering stage at the time of reaping. This was the case when the author visited the farm Oudam, Clanwilliam district. On this farm the wheat was milled in an old fashioned type of mill with no screening appliance at all. It is, therefore, possible and probable that parts of the leaves and stems of *Senecio ilicifolius* Thunb. which was found growing amongst the wheat in a corner of the land, found their way into the wheat and were responsible for the cases of suspected bread poisoning in the Smit family.

(*d*) It is quite possible that in some years the toxic weeds may be much more poisonous than in other years as variations in the toxicity of plants are well known. From the results obtained at Onderstepoort in experiments with *Senecio spp.* it appears that these plants are most poisonous in the early stages of development.

(*e*) Several poisonous weeds contaminating bread at the same time may by virtue of their synergistic effects or other actions markedly increase each other toxicity. It is, for example, quite likely that wild mustard (*Raphanus raphanistrum* L.) will, owing to its irritating effect on the gastro-intestinal tract, render *Senecio spp.* more poisonous by facilitating the passage of the active principles of these plants through the damaged mucosa.

D. Effect of the Process of Preparation of Bread in the Toxicity of Weeds Contaminating the Meal.

(*a*) *Moistening the Meal and Kneading the Dough.*

These processes will undoubtedly render the active principles (especially those soluble in water) of poisonous weeds more readily absorbable by the intestinal mucosa. Furthermore, cyanogenetic glucosides (for example vicianin contained in *Vicia sativa* L.) will be acted upon by enzymes and liberate prussic acid and those weeds which contain mustard oil compounds as active principles, for example, *Raphanus raphanistrum* L., will liberate mustard oils.

(*b*) *Baking of Bread.*

Baking is said to partly destroy the active principle of *Agrostemma githago* L., and all the evidence at our disposal seems to indicate that the active principles of *Senecio spp.* are not affected to an appreciable extent by temperatures such as are encountered in the baking of bread. As soon as fresh supplies of poisonous *Senecio spp.* are available this point will be definitely settled.

High temperatures will destroy all enzyme action thus preventing further development of prussic acid from cyanogenetic glucosides (*Vicia sativa* L.) and will at the same time expel from the bread (dough) the already liberated prussic acid.

Likewise baking will stop the liberation of mustard oils from plants containing mustard oil compounds by destroying enzyme action, and the already liberated mustard oils, being volatile, will escape from the bread during the time of baking.

Insufficiently baked bread may still contain a certain amount of the above poisonous substances which would have escaped during thorough baking or the production of which would have been rendered impossible by thorough baking.

E. THE CAUSE OF DEATH IN SENECIO POISONING.

The active principles of *Senecio spp.* must be considered primarily as liver poisons. Whether their immediate effects on the remaining organs are of any value in contributing to the cause of death is a point yet to be elucidated. Whether the gastrointestinal disturbances (inappetence, constipation, diarrhoea, ulceration of the gastric mucosa and catarrhal enteritis) are primary or secondary effects of *Senecio* poisoning is difficult to state.

In the light of our present knowledge of Senecio poisoning it appears that the funtion of the liver, especially as detoxicator and as excretor of harmful substances, is partially, or, in advanced cases of Senecio poisoning, completely destroyed. Poisonous substances, some of which are present under normal circumstances in the intestinal tract, will then be allowed to pass into the circulation and exert their harmful effects on the whole system and ultimately cause death.

Chronic *Senecio* poisoning therefore appears to cause death indirectly by destroying functions of the liver, which is the main protector of the system as far as poisoning is concerned. In acute *Senecio* poisoning there is marked destruction of the liver cells and the absorption of cell products liberated in this way will undoubtedly aid in poisoning the system.

Of interest is the following passage quoted from Wright's (1931) Applied Physiology: "The liver is thus mainly concerned with the excretion of bile pigment; the elaboration of the pigment is chiefly carried out in the bone marrow, to a less extent in the spleen and to a very slight extent in the liver (Kupfer cells)." It would, therefore, appear that in the case of a liver with an impaired function an accumulation of bile pigments in the system will occur. In high concentrations these pigments will have a detrimental effect on the system. In addition there is every reason to believe that the glycogen-glucose-lactic acid balance will be disturbed in a system with a damaged liver, the degree of disturbance depending on the degree of damage present in the liver.

V. SUMMARY.

A.—Poisonous weeds, which are liable to find their way into wheat and cause poisoning in human beings are discussed.

B.—Circumstances favouring bread poisoning, the effect of the process of preparing and baking bread on the toxicity of weeds contained in the meal, and the cause of death in *Senecio* poisoning are discussed.

C.—It would seem advisable to proclaim species of *Senecio* growing on cultivated lands as noxious weeds in addition to enforcing Regulation 12 (7) of the Food, Drugs, and Disinfectants Act, No. 13 of 1929.

VI. ACKNOWLEDGEMENTS.

I wish to record my indebtedness to Sir. E. N. Thorton, Assistant Health Officer and Director of Medical Services, Pretoria, and Dr. P. J. du Toit, Director of Veterinary Services, Onderstepoort, for their keen interest and support in this investigation; to Dr. Shanks of Humansdorp, Drs. J. Muir and Van Zyl of Riversdale, Dr. Rhodes of the Health Department, Capetown, and Dr. Truter of Citrusdal for valuable information supplied and assistance rendered by them in the course of this investigation; to Dr. G. de Kock, Deputy Director of Veterinary Services, for examining pathological specimens microscopically; to Dr. E. P. Phillips, Principal Botanist, Division of Plant Industry, Pretoria, for identifiying plant specimens and making valuable suggestions with regard to the botanical names of plants; to Mr. Groenewald, Research Officer, Onderstepoort, through whose person I managed to obtain a supply of *Senecio ilicifolius* Thunb. from a farm where cases of bread poisoning occurred; and to Mrs. J. J. du Bruyn, Modderfontein, Humansdorp district, for forwarding supplies of weed seeds for investigation.

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Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 1, Number 1, 1933.

Chemical Blood Studies.*

I. Comparative Studies on Blood, "Laked" and "Unlaked" Blood Filtrates of Animals in Health and Disease, with particular reference to methods and technique employed.

By

H. GRAF, B.Sc., D.V.Sc., Veterinary Research Officer, Department of Chemical Pathology, Onderstepoort.

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SUMMARY.
REFERENCES.

INTRODUCTION.

The present paper is intended to be the first publication of a series of researches into animal diseases occurring in South Africa. Further studies have been planned with a view to concentrating thereafter on correlating the chemical data with the pathology and pathological physiology of each particular disease in an attempt to get a rational explanation of any changes in the composition of the blood which may have been observed. The blood data are not to be restricted to the constituents enumerated in this paper, but will include in addition data on other constituents such as cholesterol, pigments, ammonia, lactic acid, mineral constituents and physico-chemical data such as viscosity, hydrogen ion concentration and sedimentation rate of cellular elements, etc. It would have been preferable to get all this information from one and the same experimental subject, but the available facilities did not permit of this.

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* "Chemical Blood Studies I, III-V" accepted as Thesis for the D.V.Sc. degree by the University of Pretoria, December, 1932. For titles of the series published to date see "References".

No serious attempt has, therefore, been made at present to offer explanations of the changes in blood composition which have been recorded, this aspect being reserved for such a time as the data referred to have been gathered. The same extensive " normal " data are being obtained for various domestic animals over a period of 12 months, those for sheep will be published by Hamersma of this Division in this Journal at a later date. Since a comparison with data obtained by other workers on normal animals is being discussed there, this aspect has been omitted. Publications on comparative data for " laked " and " unlaked " filtrates of domestic animals have not been found, in spite of a wide search through all available literature.

THE PLAN OF RESEARCH.

The present series of investigations have been primarily undertaken with a view to determining the actual changes, if any, occurring in the composition of the blood during the course of a number of different protozoan and virus diseases. The conditions investigated up to the present are: (1) Heartwater of sheep (*Rickettsia ruminantium* infection), (2) Horsesickness (*Pestis equorum*), (3) Anaplasmosis of cattle (*A. marginale* infection)), (4) Piroplasmosis of cattle (*P. bigeminum* infection), (5) Bluetongue of sheep, and (6) Anaplasmosis in the Blesbok (*Damaliscus albifrons*). For the results of these investigations up to the present see " Chemical Blood Studies III-V " in this Journal. Such data in respect of the diseases studied, have up to now, been completely wanting in South Africa, and in spite of an exhaustive search in the available literature no chemical data on the conditions detailed here could be found. It was felt that such data may materially contribute towards a deeper understanding of the pathology of these diseases, also enabling a clearer conception to be formed of the processes taking place in the body as a result of such specific infections. It was also anticipated that these researches, apart from increasing our knowledge as to the actual changes in the composition of the blood during infectious conditions, may become of value from a diagnostic, prognostic or prophylactic point of view.

No search was made for constituents not normally occurring in the blood, but which may possibly be present as a result of the abnormal metabolism of the body under the stimulus of the causal agent or its excretory products, or secondary stimuli associated with the symptoms of the disease such as hyperexia, anaemia, anorexia, etc.; or which may constitute the toxins themselves or the by-products of the metabolism of the causal agent. The aim was rather to note the changes in the relative proportions of certain normal constituents during any particular infection.

Determinations in respect of the total nitrogen (T.N.) and haemoglobin content (Hb) of the whole blood, and non-protein nitrogen (N.P.N.), urea nitrogen (U.N.), amino-acid nitrogen (A.A.N.), uric acid nitrogen (U.A.N.), total creatinine nitrogen (T.C.N.), and sugar (S) were, therefore, made in each case on both " laked " and " unlaked " blood filtrates, which were prepared according to the method of Folin and Wu (laked) and Folin (unlaked). " Laked " and " unlaked " filtrates were studied in order to obtain figures comparable with those obtained elsewhere during similar investigations into animal diseases, whether done on either filtrate. In view of the unequal distribution of most constituents over the plasma and the cellular elements, a study involving both types of filtrates was an additional inducement to undertake the large amount of extra labour involved in analysing two filtrates of one and the same blood.

Owing to the absence of normal figures for ovines, bovines and equines for the above constituents under South African conditions, several blood analyses were made prior to infecting the experimental subject. In a few cases where this was not found possible, analyses were made on the day of infection, and in some rare cases even a day or two later.

DIET OF EXPERIMENTAL ANIMALS.

No special diet was given, the rations being in all cases those supplied to the stock at this Institute. Sheep received 1 lb. of mealies, veld hay and green feed (when available) *ad lib.*, plus $\frac{1}{8}$ ounce of salt per day; cattle received 2 lb. mealie meal, 2 lb. mealie bran, veld hay and green feed (when available) *ad lib.*, plus 1 ounce of salt per day. Horses received 5 lb. of mealies, veld hay and green feed (when available) *ad lib.*, plus $\frac{1}{2}$ ounce of salt per day. Sick horses received 6 lb. of mealie bran and 2 lb. of crushed oats instead of the 5lb. of mealies. The green feed consisted of either lucerne, green barley or oats. The food was given at 7 a.m. and 4.30 p.m.; water three times a day. As the nature and amount of the diet influences to some extent the composition of the blood, the system of analysing the blood repeatedly before and after infection of one and the same animal on a " fixed " diet, eliminates largely the complication of the influence of the diet on composition. In this connection it should also be borne in mind that during severe hyperrhexias, especially during the critical period, animals not uncommonly refuse food—this anorexia *per se* influencing the composition of the blood.

As, however, such an anorexia constitutes a part of the symptom complex, its influence on the composition must be regarded as abnormal and as part and parcel of any pathological changes which may be observed.

It was not considered of sufficient value to determine accurately the intake of food by each individual animal, since the present work is not so much concerned with how the food influences the blood composition but rather a study of the influence on the body of various infections as reflected in the blood. This system also furthermore permits of seasonal variations in the normal composition of the blood to be taken into consideration, whether due to diet, environmental temperature, humidity, etc., or not. It, therefore, allows for an accurate comparison of the figures obtained for the various constituents in health and during any particular infection.

The subjects were in all cases placed on temperature for long periods prior to infection and clinical examinations made whenever it was deemed necessary for the purposes of these investigations.

TECHNIQUE AND METHODS.

Full reference to the origin of the methods here used are given under the various sub-sections and only where modifications of any given method were introduced, or special points of interest emerged, have these been detailed here. In all cases such changes of the prescribed procedure have been thoroughly checked before being adopted for the analyses of blood filtrates.

(1) Method of taking Blood Samples (Neser, 1923).

The blood was in all cases drawn from the jugular vein with sterilised trocar and canula or hollow bleeding needles and collected in 30-35 c.c. vaccine bottles containing 0·25 c.c. of a 20 per cent. potassium oxalate solution. These bottles were filled with blood so that less than one c.c. of oxalate per 100 c.c. blood was used. This amount of anticoagulant proved sufficient, except in the case of sheep blood, with which occasional clotting took place, such bloods having then to be discarded. The bleeding took place, in the majority of cases, between the periods 8.30 a.m.–9 a.m., except where otherwise stated. This enabled me to collect as nearly as possible comparative data as far as the period between the morning feeding of the stock and the withdrawal of the blood was concerned, and further permitted the analyses being completed the same day (except the amino-acid nitrogen determinations, since with these the colorimetric readings had to be delayed for 24 hours). Serial analyses could thus be made without difficulty.

In order not to introduce a complicating factor through the production of an anaemia as a sequel to too frequent bleedings, bleedings were undertaken with as long intervals as the main objects of this research permitted. The analyses were begun immediately, precipitation of the proteins being started within a few minutes of bleeding.

(2) Preparation of Blood Filtrates.

As previously stated, determinations were made on so-called " laked " and " unlaked " protein-free filtrates, the method followed being in both cases those advocated by Folin and Wu (1919), and Folin (1930), i.e. using tungstic acid as the protein precipitant.

(*a*) " *Laked* " *Blood Filtrates.*—The addition of 10 c.c. of blood to 70 c.c. of distilled water in a 100 c.c. container was followed by this mixture being well shaken to permit of thorough laking. 10 c.c. of 11 per cent. sodium tungstate solution ($Na_2WO_4{\cdot}2H_2O$) were now added, followed by 10 c.c. ·725 per cent. normal sulphuric acid, the mixture being again well shaken. With all samples having an approximately normal haemoglobin content, the colour changes in the mixture are those described by Folin and Wu, the higher the haemoglobin content, the darker is the brown colour of the precipitate, but with anaemic bloods the colour is of varying depths of dirty pink, the more anaemic the lighter the colour remains.

Folin and Wu advocate the use of a 10 per cent. sodium tungstate solution and ·66 N sulphuric acid, but the author has encountered blood specimens where, when using these concentrations, the filtrate either did not come through water clear or came through clear but later became slightly turbid, especially was this the case with equine blood. When using the slightly higher concentrations, clear filtrates were always obtained.

On making check determinations with filtrates obtained with 10 per cent. and 11 per cent. sodium tungstate solutions for all the constituents, no differences in the results—within the experimental error—could be noted. During filtration—through Schleicher and Schüll's folded filter paper No. 588, 15 cm. diameter—the first few c.c. of filtrate were always returned. The filtrate is only very slightly on the acid side, except with anaemic blood, in which case it is distinctly more acid.

Ten c.c. blood yields sufficient filtrate for duplicate determinations of all the above-mentioned constituents.

In view of the number of different constituents which are determinable in the filtrate, this method of precipitation offers undoubted advantages and must be regarded as a notable advance in the technique for the study of such a complex tissue as blood.

(*b*) "*Unlaked*" *Blood Filtrates.*—For these Folin's (1930) method was employed, except that filtration, instead of centrifugation was used, no trouble being experienced in getting sufficient filtrate before disintregration and haemolysis set in. The filtration was carried on only sufficiently long to obtain sufficient filtrate for the determinations—generally about 30 minutes—the darkening of the blood during this period being only slight.

With the majority of blood samples visible disintregration, except darkening of the precipitate which takes place earlier, occurs only about after an hour, except in the case of bovine blood, which haemolyses more rapidly. In very rare cases disintegration occurred within 20-25 minutes with some pathological samples. Anaemic blood samples can immediately be spotted by their paler pink colour after the addition of the $\frac{1}{3}$ N sulphuric acid. In the case of the laked filtrate there is undoubtedly the objection raised by Wu (1922), that constituents of the disintegrated blood corpuscles are included, but where both filtrates are used concurrently, this very fact is of some interest in indicating in which fraction, i.e. whether in the cellular or plasma fraction, the constituents are concentrated, and whether any changes in the normal relative proportions can be noted under pathological conditions. This aspect will again be referred to in the final discussion of the data obtained.

(3) AMINO-ACID DETERMINATIONS.

Folin's (1922) method was used, utilising 10 c.c. of each filtrate. The colorimeter was set generally at 10 mm. and no trouble in reading experienced except where the amino-acid content was found to be relatively low. In such cases the colour tint of the unknown tended more towards a yellow, and exact matching became virtually impossible. No efforts were made to attempt to determine why the colour in such cases differed from the standard. The cause may be the " dilution phenomenon " as suggested by Folin (1922) or be associated with a change in the relative proportions of the various amino-acids composing the " amino-acid nitrogen " fraction of the blood, when the total " amino-acid N ", as given by this method, is low. Folin draws attention, during the discussion of the method, to the fact that only a part of the nitrogen, i.e. the nitrogen in the -NH_2 grouping reacts with the quinone reagent and that, therefore, certain amino-acids give readings which are in reality too low. He instances, amongst others, histidine which only reacts with one-third of its nitrogen, and tryptophane reacting with one-half. It is, therefore, theoretically at least, possible to obtain a low reading, even although the total amino-acids actually present may be relatively high. Under normal conditions the actual proportions and number of different amino-acids circulating in the blood is probably fairly constant, subject to the influence of the diet, but during pathological conditions especially septicaemias, selective destruction by the micro-organisms of one or more amino-acids would tend to disturb the normal proportions.

Unfortunately our knowledge of the metabolism of the micro-organisms in biological fluids or tissues intravitam during any specific infection, what they live on, what products are excreted by them, and what changes, if any, are brought about by these excreted substances in the surrounding medium, is extremely limited at present.

(4) URIC ACID DETERMINATIONS.

The " uric acid N " in both " laked " and " unlaked " filtrates was determined by Folin's 1930 method It was, however, found to be more satisfactory in the majority of cases, especially with sheep blood, where the uric acid concentration is low, to make the volume up to 15 c.c. instead of 25 c.c. for the colorimetric readings.

(5) THE DETERMINATION OF " UREA N."

For this Folin and Svedberg's urease (1930) method [see also Folin and Denis (1916)] was employed and once the technique had been acquired was found to be simple and convenient. At the beginning, however, difficulty was experienced in obtaining uniform results when making a series of duplicate determinations on solutions of urea and blood filtrates to which known amounts of urea had been added. Jorden and Graf (1933) of this Institute, investigated the method, and after a thorough check of all the reagents and the technique employed, suggested that possibly the fault lay with the amount of buffer solution added to the unknowns. Further detailed work in this connection confirmed this suspicion. It was found that constant results were obtainable through the addition of more buffer solution, the optimum being determined to be 1·5 c.c. instead of the two drops recommended by the authors of the original method. Full details of this work are given in this (1933) Journal (see pages 279-283). In my determinations I have used 1·5 c.c. of the buffer solution and have obtained excellent results. For the preparation of the urease paper extracts made from locally grown soya beans and from " Merck's Soya bean meal ", gave equally satisfactory results. After the addition of the urease paper, the tubes were allowed to stand for 1-1½ hours, with occasional shaking. The small anti-bumping tubes not being available, a small glass bead was substituted and no trouble experienced with bumping once the technique of the urea distillation had been mastered. In most cases a standard corresponding to 10 mgm. urea nitrogen per cent. was employed owing to the low blood urea often encountered, although the 20 mgm. per cent. standard was also always made up. With high urea-containing filtrates, dilutions were made in such a way as to give colours after nesslerisation, closely approximating the colour of the standard. Repeated blanks were made throughout the course of these investigations, and in consequence an average figure of 1 mgm. urea N per cent. was subtracted from the " urea N " figures obtained.

(6) NON-PROTEIN NITROGEN.

Folin and Wu's (1919) method [see also Folin and Svedberg (1930)] was used and all determinations made in duplicate, two standards in each case being made up, containing ·3 and ·15 mgm. N respectively. Where the N.P.N. was high, the determination was repeated with smaller amounts of filtrate, so that the colour obtained would approximate the standard solution. It is of great importance to continue the micro-digestion sufficiently long to ensure complete digestion, otherwise the readings will be too low. I have found the best results are obtained if the digestion is carried so far that when 15-20 c.c. of water are added, the solution is very slightly turbid. This turbidity disappears on making up to the final volume of 50 c.c. If the digestion is carried further, even for only a few seconds, a marked turbidity frequently results, which then interferes with accurate colorimetric readings, results which are too high being obtained. With experience the correct degree of digestion can be readily acquired.

(7) " TOTAL " CREATININE DETERMINATIONS.

Folin and Wu's (1919) method was used, the picric acid being purified with the method of Benedict (1929) from glacial acetic acid. In connection with the method as described on page 100 of the Journal of Biological Chemistry, Vol. 38, 1919, an error in the calculation has crept in. Instead of multiplying by 6 it is necessary to multiply by 12, since 20 c.c. of the standard solution recommended contain ·12 mgm. of creatinine. With 10 c.c. of the standard solution (Hawk, 1931), the calculation as given, would be correct.

When accidental overheating in the autoclave occurred a turbidity due to the presence of a white precipitate necessitated a repeat determination.

(8) Total Nitrogen (on Whole Blood).

For this 1 c.c. of oxalated blood was digested in a Kjeldhal flask with 15 c.c. of sulphuric acid-copper sulphate (15 c.c. acid and 1 c.c. 6 per cent. copper sulphate) digestion mixture until clear, the ammonia being distilled into a known volume (25-35 c.c.) of ·1 N sulphuric acid. A blank of 20 mgm. was allowed for.

(9) Haemoglobin.

One c.c. of whole blood was diluted to 200 c.c. with ·1 N hydrochloric acid to convert the haemoglobin into acid haematin. Full development of the colour is not obtained at once, all readings being taken with day-light illumination four hours after dilution. The standard employed was a Newcomer (1919) disc which had been standardised in this laboratory against the van Slyke (1921, 1924, and 1927) gasometric haemoglobin determination method.

The colorimetric readings were then converted by means of tables supplied with the disc into " haemoglobin per cent. Williamson's Standard " and this reconverted into " Grams Haemoglobin per 100 c.c. blood ". No correction was made for the small amount of anti-coagulant used—less than 1 per cent. All precautions such as detailed in Fourie's (1931) paper, viz. thorough shaking of the acid haematin solution, absence of gas bubbles in the pipette, etc., were taken into account.

(10) Sugar Determinations by Folin's (1929) Method.

In connection with this method the special Folin-Wu sugar tubes were not available in time and in their stead 18 mm. diameter test tubes 21 cm. long had to be utilised, only this type of test tube being used throughout. With them more constant results were obtained when the heating was continued for 20 minutes instead of the 14-15 minutes recommended. No time should be lost in the cooling after the heating and the addition of the acid molybdate reagent. Thorough mixing is essential. Owing to the relatively low blood sugar content of some animals, particularly sheep, only 1 c.c. standard glucose solution had frequently to be used, the final volumes being made up to 15 c.c. instead of 25 c.c.

(11) The Use of the Colorimeter.

For all the colorimetric work a " Holri " 50 mm. E. Leitz colorimeter was utilised. Particular attention was paid to noting zero points, setting the two fields evenly, interchangeability of cups, etc., in order to obtain accurate readings. As a general rule I preferred setting the standard at 10 mm., except for abnormally dilute solutions. With the lighter tints of the fields obtained in this way it appeared easier to get more constant readings, the difference in tints being more readily noticeable. 2-3 Careful readings were taken for each unknown, more readings throwing an undue strain on the eyes and tending to inaccuracy rather than accuracy. The source of illumination was always daylight against a white background.

(12) TEMPERATURE CHARTS.

In order to indicate the type of temperature reaction, and more particularly to demonstrate at what periods of the reaction blood examinations have been made, charts have been incorporated. The periods at which blood has been drawn has been indicated on the curves. The temperatures were taken twice daily (once only on Sundays) at 6.30 a.m. and 3.30-4 p.m. respectively. Only the actual reactions are recorded, the normal temperature records being omitted for the sake of economy of space, no useful purpose being served by the incorporation of several weeks of such normal records.

(13) TABLES OF ANALYTICAL DATA.

These are mostly self explanatory, all constituents being expressed in " mgm. per 100 c.c. of blood " except haemoglobin and total nitrogen, both of which are expressed as " grams per 100 c.c.". The " coaguable nitrogen " has been obtained by calculation (Total N - N.P.N.). The " Rest nitrogen " represents the nitrogen fraction unaccounted for in any specific form after the " urea N ", " Total creatinine N ", " uric acid N ", and " Amino acid N " had been subtracted from the " Non-protein N " figure. The urea (46·66 per cent. N), total creatinine (37 per cent. N) and uric acid (33·33 per cent. N) have for the sake of convenience been expressed both as such and as " Nitrogen ". In the column " plasma " the symbols " n.u." (nothing unusual) refer to the physical appearance of the plasma, more particularly to its colour. In anaplasmosis, redwater and horse-sickness, icteric plasmas were quite frequently encountered. The " plasma " column has been omitted in the case of heartwater, since the plasma at no time showed any haemolytic or icteric discolouration.

In the column " Temperature Reaction " the following symbols have been adopted:—

N (Normal) - meaning that no abnormal temperature reaction is going on at the time of bleeding.

P.I.N. (Post infectionem, normal) = indicating that the animal has been injected with virus, but that as yet no temperature reaction has set in.

R (Reaction) - indicating that blood was withdrawn during the course of a reaction.

" Time of bleeding " = where no symbol is given it means that the blood was drawn between 8.30 a.m. and 9.30 a.m.

In other cases the time of bleeding is inserted. The above system has been applied throughout these publications.

SUMMARY.

A scheme has been outlined of a series of researches into various animal diseases and the technique and the chemical methods utilised have been described. The present paper is to be regarded as the first of a series of publications to be issued under the general title of " Chemical Blood Studies " and is to serve as a general introduction for the series. The chemical determinations include total nitrogen (T.N.), haemoglobin (Hb), " Total " creatinine nitrogen (T.C.N.), urea nitrogen (U.N.), uric acid nitrogen (U.A.N.), amino-acid nitrogen (A.A.N.), sugar (S.), and non-protein nitrogen (N.P.N.).

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GRAF, H. V. Comparative studies on "laked" and "unlaked" blood filtrates of cattle in health and during Anaplasmosis and Piroplasmosis.

HAMERSMA, P. J. VI. A serial study (over a 12-month period) of some organic constituents in "laked" and "unlaked" blood filtrates of healthy sheep of various ages. To be published in subsequent number of this Journal.

Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 1, Number 1, 1933.

Chemical Blood Studies.*

II. A contribution to the determination of Urea in Animal Blood Filtrates ("Laked" and "Unlaked").

By T. J. WILKEN-JORDEN, D.Sc., Dip Research Chemist, and H. GRAF, B.Sc., D.V.Sc., Veterinary Research Officer, Department of Chemical Pathology, Onderstepoort.

In the course of studies (Graf, 1933) on the composition of blood of domestic animals subjected to various South African stock diseases it was found that the method of Folin and Svedberg (1930) for the determination of urea gave most inconsistent results. For the precipitation of the protein matter in the laked blood 10 c.c. of a 0·725 N sulphuric acid and 10 c.c. of a 11 per cent. sodium tungstate solution were used, as against 10 c.c. of a 0·66 N sulphuric acid and 10 c.c. 10 per cent. sodium tungstate as prescribed by Folin and Wu (1919). This change became necessary since it was found that with some blood samples the proteins were not completely precipitated when using the prescribed amounts of reagent. The unlaked blood, on the other hand, was treated strictly according to standard procedure. After various preliminary experiments, and after checking up the reagents employed most carefully, only two possible sources of error remained to be considered, viz. :—

I. That the degree of acidity of the blood filtrates was such that the 2 c.c. of saturated borax solution employed was not sufficient to liberate on distillation all the ammonia derived from the urea. It should be pointed out here that, on account of the presence of amino-acids and other amino-derivatives contained in the blood filtrate, no strong alkali can be used for liberating the ammonia, as amino substances would thus be subjected to partial hydrolytic decomposition with the liberation of additional ammonia.

II. —That, as a result of the work by other investigators on the activity of the urease enzyme, and more specially as the result of the valuable study by Barendrecht (1919) concerning the effect of hydrogen ion concentration on this activity, it became evident that too great a fluctuation in the pH of the medium would result in erroneous urea determinations. In Folin and Svedberg's method it is suggested to regulate this apparently fluctuating degree of acidity of medium by buffering with two drops of acetate buffer.† Whether by this means an optimum pH for urease activity will be achieved obviously depends on the degree of acidity (or alkalinity) of the initial blood filtrate.

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* The titles of the series will be found under "References."

† Dissolve 15 gm. of crystallized sodium acetate in a 100 c.c. volumetric flask by the help of 50 to 75 c.c. of water. Add 1 c.c. of glacial acetic acid (about 99 per cent.), dilute to volume, and mix.

Measuring the pH of such animal blood filtrates by the potentiometric method, using the quinhydrone electrode, it was found that these filtrates varied appreciably in pH, although always distinctly acid in reaction. In the table below are given the limiting values of pH of blood filtrates obtained from blood drawn from normal and infected animals of different species.

Animal.	Number of cases.	Laked or unlaked.	pH Fluctuation.
Sheep..........................	20	Laked..........	3·01—4·30
	24	Unlaked.......	3·75—4·59
Bovine.........................	8	Laked..........	2·98—4·13
	8	Unlaked.......	3·79—5·06
Blesbok (*Damaliscus albifrons*)...	6	Laked..........	2·71—3·69
	6	Unlaked.......	3·81—4·23

It would appear, therefore, that the acidity of blood filtrates varies greatly, ranging at least from pH 2·71–5·06.

I.—THE LIBERATION OF AMMONIA BY MEANS OF BORAX.

In order to investigate the first possible source of error, a series of solutions of different pH and containing 2·5 c.c. of a standard solution of ammonium carbonate (5·0 mgm. N/100 c.c.) was prepared by adding varying quantities of dilute sulphuric acid (N/20) and diluting up to a final volume of 8 c.c. In all cases the two drops of acetate buffer solution, as recommended by Folin, were added. The pH of these solutions, and the amounts of ammonia recovered after distilling with borax and Nesslerization have been tabulated in Table I.

TABLE I.

	c.c. N/20 H_2SO_4 added.	pH of Soln. with buffer.	Borax Soln. added.	pH of Soln. after adding borax.	NH_3 found as N.
1........	0·0 c.c.......	5·45	2·0 c.c..	ca. 9·2	0·138
2........	0·2 „	5·25	2·0 „ ..	„ 9·2	0·138
3........	0·6 „	5·03	2·0 „ ..	„ 9·1	0·147
4........	1·0 „	4·81	2·0 „ ..	„ 9·0	0·138 *Blank=*
5........	2·0 „	4·20	2·0 „ ..	„ 8·9	0·138 0·013
6........	3·0 „	2·76	2·0 „ ..	„ 8·8	0·138 *mgm N*
7........	4·0 „	2·37	2·0 „ ..	—	0·147
8........	3·0 „ N/10	1·90	2·0 „ ..	—	0·092
9........	3·0 „ N/10	1·90	4·0 „ ..	—	0·100

It is clear, therefore, that down to a pH of 2·4 the 2 c.c. of borax solution added is sufficient to liberate all the ammonia quantitatively. As it is also highly improbable that the acidity of a blood filtrate will rise to a pH below this limiting value, it may be concluded that the 2 c.c. of saturated borax solution added will in every instance liberate quantitatively all the ammonia present as such at the time of distillation.

II.—The Regulation of pH and Urease Activity.

Having shown that the 2 c.c. borax solution proved adequate for the final liberation of the ammonia, attention was next diverted to the urease conversion of the urea into ammonium carbonate. The urease used was derived from the Soja or Jack bean by grinding up the beans, and extracting in the cold with ca. 30 per cent. aqueous alcoholic solution using 100 c.c. of this diluted alcohol per 30 gm. soja-bean meal. The extract was absorbed by filter paper (NH_3-free) which was then dried and cut up into rectangular pieces approximately 1 × 2.5 cm.

For the purpose of studying the effect of pH on the activity of the urease, a series of solutions of varying pH and containing 2·5 c.c. of a standard urea solution (5·0 mgm. N/100 c.c.) was prepared by adding the necessary amount of N/20 sulphuric acid, the two drops of acetate buffer, and diluting to a final volume of 8 c.c. To each of these solutions two pieces of the urease paper were added and the solutions allowed to stand from two to four hours with occasional shaking. The results, obtained in duplicate, have been tabulated in Table II.

Table II.

Experiment.	c.c. N/20 H_2SO_4.	pH of sol. without buffer.	pH of sol. with buffer.	Reaction time with urease.	c.c. Borax added.	NH_3 found, as mgm. N.	
						Duplicate values.	Average.
1.......	0·0 c.c.	ca. 8·0	5·45	2 hrs. 5 min.	2·0 c.c.	—	0·128
2.......	0·5 ,,	,, 2·54	5·05	2 ,, 25 ,,	2·0 ,,	0·125 0·128	0·127
3.......	1·0 ,,	,, 2·30	4 81	3 ,, 5 ,,	2·0 ,,	0 121 0·115	0·118
4.......	2·0 ,,	,, 2·03	4·20	3 ,, 15 ,,	2·0 ,,	0 080 0·100	0·090
5.......	3·0 ,,	—	2·76	3 ,, 35 ,,	2·0 ,,	—	0·014
6.......	4·0 ,,	,, 1·81	2 37	3 ,, 55 ,,	4·0 ,,	0·015 0·011	0·013
7.......	3·0 ,, N/10	,, 1·66	1·90	4 ,, 15 ,,	4 0 ,,	0·012 0·011	0·012

From these results it would appear that in experiments 5–7 the activity of the urease is zero, since the ammonia found corresponds almost exactly with the blank values derived from Table I. However, the marked effect of pH on the activity of the urease is best illustrated in the accompanying graph, obtained by plotting the weight (in mgm.) of recovered N against the pH of the medium. We note that with an acetate buffer the urease activity reaches an optimum at an acidity within the pH limits 5·0 and 5·5. Below pH 5·0 there is a very sudden fall in activity resulting ultimately in stagnation. It is clear, therefore, that the urease can effect a quantitative conversion of urea into ammonium carbonate only if the acidity of the medium is carefully regulated and kept within the optimum pH range of 5·0 to 5·5.

From Table II it is also evident that the addition of two drops of acetate buffer does not necessarily result in the attainment of this optimum condition. On the other hand, the pH of the buffer solution itself must lie somewhere near 5·5, since such solutions do not appreciably alter in pH on dilution. Hence the pH of all solutions, irrespective of their initial acidity, must eventually be brought to fall within this optimum range if sufficient buffer solution is added. That this desired effect can easily be achieved in the actual course of analysis is shown by the results tabulated in Table III.

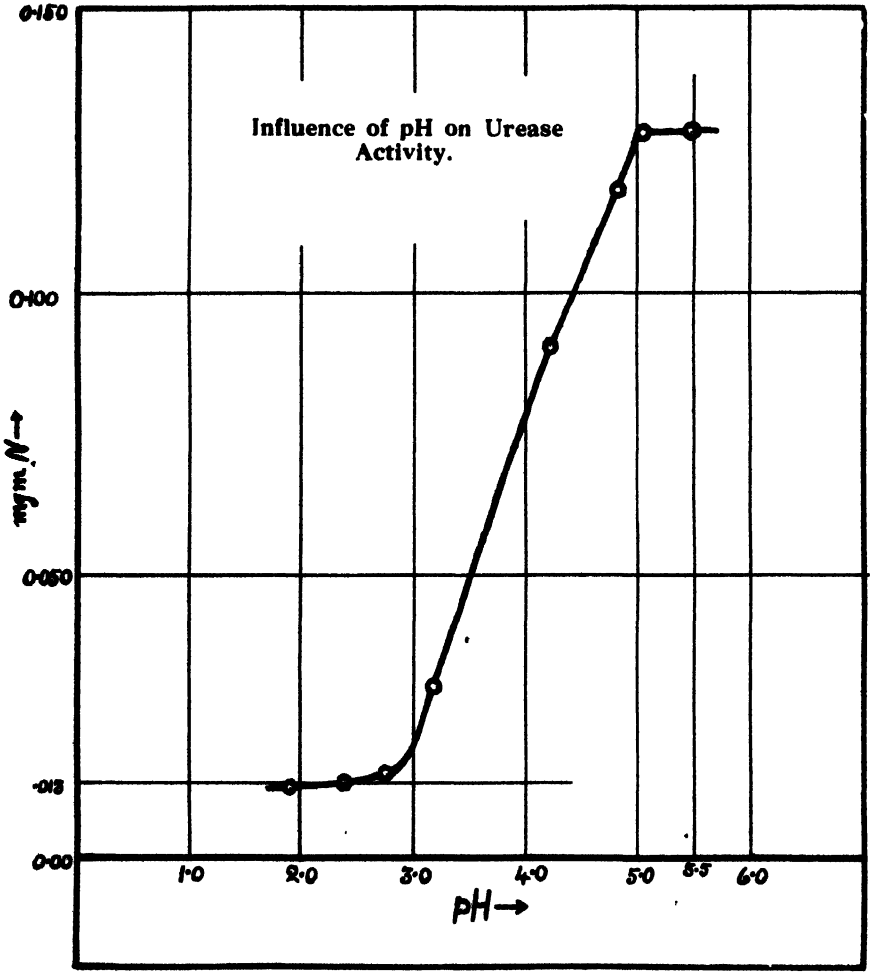

TABLE III.

c.c. n/20 H_2SO_4 added.	Initial pH (o.o. c.c. buffer).	pH 2 drops buffer.	pH 4 drops buffer.	pH 8 drops buffer.	pH 10 drops buffer.	pH 20 drops buffer.
0·0 c.c.	ca. 8·0	5·45	—	—	5·43	5·37
2·0 „	„ 2·03	4·20	4·67	4·95	5·05	5·15
4·0 „	„ 1·81	2·37	—	—	4·81	5·05

The solutions used in the above experiment were obtained by adding the specified amounts of n/20 sulphuric acid to 2·5 c.c. of the standard urea solution, then adding the required amount of buffer solution and finally diluting to 8 c.c. Hence, starting with an initial pH as low as 1·8 and adding 20 drops of the acetate buffer, the pH of the resulting medium is brought to within the required optimum range. Again, as the highest acidity found in blood filtrates was of the order of pH 2·7, it may be concluded that the addition of 20 drops buffer solution should prove adequate in all cases. Folin and Svedberg's (1930) urea determination method must therefore be modified if serious errors are to be avoided. It is suggested that instead of two drops of acetate buffer, 1·5–2·0 c.c. of this buffer solution be used per 5 c.c. blood filtrate.

Applying the method in its thus modified form, and using 1·5 c.c. buffer solution per 5 c.c. aliquot blood filtrate, good results are obtained as Table IV readily shows.

TABLE IV.

Animal.	Laked or unlaked.	pH of blood filtrate.	Urea found as mgm. n/100 c.c. blood.
Sheep No. 26689..........	Laked..........	3·63	20·0
	Unlaked........	4·27	19·4
Bovine No. 3532..........	Laked..........	3·93	12·2
	Unlaked........	4 31	11·4
Blesbok No. 32055.......	Laked..........	2·71	12·6
	Unlaked........	3·81	12·2
Blesbok No. 32054.......	Laked..........	3·45	19·0
	Unlaked........	3·83	17 4

Since this modification was introduced hundreds of urea determinations have been made in this laboratory in the course of studies (Graf, 1933) on animal blood. In no case was any further difficulty encountered.

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JORDEN, T. J., AND GRAF, H. II. This article.

GRAF, H. III. Comparative studies on "laked" and "unlaked" blood filtrates of sheep in health and during Heartwater and Bluetongue.

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GRAF, H. V. Comparative studies on "laked" and "unlaked" blood filtrates of cattle in health and during Anaplasmosis and Piroplasmosis.

Onderstepoort Journal of Veterinary Science and Animal Industry, Volume I, Number 1, 1933.

Chemical Blood Studies.*

III. Comparative Studies on "Laked" and "Unlaked" Blood Filtrates of Sheep in Health and during "Heartwater" (*Rickettsia ruminantium* infection) and Bluetongue (Catarrhal fever).

By H. GRAF, B.Sc., D.V.Sc., Veterinary Research Officer, Department of Chemical Pathology, Onderstepoort.

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III. GENERAL SUMMARY.

IV. ACKNOWLEDGMENTS.

V. REFERENCES.

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* "Chemical Blood Studies," I, III-V was accepted as Thesis for the D.V.Sc. degree by the University of Pretoria, December, 1932. For series of articles published up to date see "References."

I.—GENERAL INTRODUCTION.

The present article is the third of the above series (Graf 1933) and deals exclusively with chemical research work in connection with Heartwater and Bluetongue. It has for a long time been felt that the absence of biochemical data, particularly in respect of the blood constituents in diseases of domestic animals, has been a great drawback from the veterinarians and veterinary pathologists points of view. A clear conception of the pathology is not possible without a knowledge of the chemical processes involved. It is towards this aspect that these researches are ultimately directed. In order to avoid needless repetition, the objects, technique and the methods employed have been fully detailed in the first article (Graf 1933), page 269, in this Journal.

II.—A. HEARTWATER IN SHEEP.

It is beyond the scope of this paper to discuss in detail the distribution, symptomatology, pathology, etc., of this disease, but for those to whom this condition is unknown, a short note may be of interest. For a comprehensive review of our present state of knowledge in heartwater in all its aspects, I would refer to the most recent publications on this disease, viz.: by Alexander (1931), in which is included a full bibliography, and that by Jackson and Neitz (1932).

Heartwater may be defined as "a febrile septicaemic infectious disease of sheep, goats and cattle, caused by *Rickettsia ruminantium*, and transmitted by ticks of the genus *Amblyomma*, chiefly *A. hebraeum*." The disease receives its name from the common pathological lesion in sheep and goats, namely, a well marked hydropericardium, though this is not a constant nor the most pronounced finding in cattle. Animals which recover do not harbour the virus in the blood, which is infective during and for a short time after the reaction. Recovery from a reaction results in a solid, but not absolute immunity." (cit. Alexander.)

The disease can be readily produced in susceptible animals by the injection of 5–10 c.c. blood taken from a reactor during the actual time of reaction and for a short while thereafter. The incubation period is from 5–35 days, generally 7–14 days.

The symptoms of the disease cannot be regarded as pathognomonic. There is usually a sudden rise in temperature, which may go up to 108°, followed by gradual loss of appetite and cessation of rumination. Associated with the hyperthermia there is usually dyspnoea, rapid pulse, becoming weaker with the progress of the disease, cyanosis of the mucous membranes and nervous symptoms gradually supervene, the latter being present in the majority of cases. The nervous symptoms usually show in the form of unsteadiness of gait, unnatural position when standing or lying, when down galloping movements of legs, champing of the jaws with resultant frothing. The agonal period may extend up to 24 hours. The mortality varies considerably, being for Merino sheep about 60 per cent., in the present experiment 75 per cent.

At post-mortem few really characteristic lesions are found, but there is commonly cyanosis of the mucous membranes, hydroperitoneum, hydropericardium, subendocardial haemorrhages, cloudy swelling and fatty degeneration of the myocardium and oedema of the lungs, swelling, hyperaemia, fatty degeneration and bile stasis of the liver, hyperaemia and degeneration of the kidneys.

The histo-pathology of the disease has been studied by Steck (1928), who summarises his findings as follows: "Besides the presence of *Rickettsia ruminantium* (Cowdry) the characteristic changes in heartwater are leucostasis and perivascular cellulation. The former occurrs in all organs and the macrophage is the prominent cell, but lymphocytes and neutrophiles are also numerous. The latter is pronounced in the liver and kidneys and sometimes in the adrenal glands.

All the cells of the mesenchyma may take part in this reaction, but mainly those of the lymphocytic series, viz.: lymphocytes and plasma cells. It seems most likely that these alterations are due to a noxe which is spread diffusely by the blood stream. It must remain for further investigation to determine the nature of this noxe."

(*a*) SELECTION AND TREATMENT OF THE EXPERIMENTAL SHEEP.

In these researches sheep (Merinos) were specially selected from among a large herd purchased in heartwater-free areas of South Africa to ensure their being susceptible. Clinical examinations were made to ensure getting normal healthy sheep in good condition. Faecal examinations to determine the degree of helminthic infestation were also undertaken (through the courtesy of Dr. Mönnig). Only sheep with very light infections were utilised after being dosed with Wireworm Remedy, which dosing was furthermore carried out monthly.

The success of these precautions was amply demonstrated at the post-mortem examinations where, with the exception of a few oesophagostome nodules, no adult worms could be found in any of the cases. Fourie (1931) reports on the morphological changes associated with haemonchosis, changes which probably also affect the chemical composition, and it was primarily to reduce or virtually eliminate such complications that the reduction of the degree of verminosis was undertaken. The sheep in all cases were placed on temperature for varying lengths of time before injection with heartwater virus, several different strains of it, including natural or veld strains being used. To the virulency of the strains employed, the short duration of the disease in most cases and the heavy mortality, 75 per cent., bear testimony. Only three of the twelve cases recovered.

(*b*) CHEMICAL METHODS AND TECHNIQUE.

For these see Chemical Blood Studies I (Graf 1933) (this Journal).

(*c*) EXPERIMENTAL DATA.

In the preparation of this paper some difficulty was experienced as to what would be the most convenient method of presenting the large mass of data collected so as to bring out those features differing from the normal. The system finally decided upon was to briefly draw attention to the salient features in each case of heartwater emerging from the analytical data, treating each constituent separately and thereafter summarising the findings in such a way as to focus attention on the deviations from normal more or less common for all the cases studied.

As it is essential to know first the normal range of variation for each constituent before it can be known what is abnormal or pathological, the analytical figures obtained before the injection of virus have been collected and summarised. The numbers presented here are relatively few, but when discussing bluetongue, further normal data will be presented. During the last year detailed investigations on similar lines on normal healthy sheep have been carried out by Hamersma (1933) of this Division, and these will be published in the next number of this Journal (*vide* Chemical Blood Studies VI).

(1) *Normal Range of Constituents of Blood Filtrates.*

Sugar.—This was found to vary from 32·90 to 73 mgm. per cent. in the case of " laked " filtrate, the figures falling in the majority of cases between 45–55 mgm. per cent. In the " unlaked " filtrates the variations ranged from 26·20–68·50 mgm. per cent. with the majority of figures lying between 31–42 mgm. per cent. The amount of sugar determined was in all cases lower in the " unlaked " than in the " laked " to the extent of 15–40 per cent., with an average of approximately 25 per cent. In one case the difference was as little as 2 per cent., in another as much as 60 per cent.

Total Nitrogen.—The variations found ranged from 2·2–2·9 gm. per cent., both these figures being, however, only once determined, by far the greatest number falling within the 2·3–2·6 gm. per cent. range—a remarkably constant and relatively narrow range. This is even more striking when the considerable variation in the haemoglobin content of the blood is considered, not only of the same animal at different bleedings, but also in the different animals when compared with each other.

Urea Nitrogen.—When summarising the " Urea N " content of the " laked " filtrates before injection of virus, figures varying from 3·42 mgm. N per cent. to 20·45 mgm. N per cent. (7·14–42·84 mgm. urea) are encountered. The following tables illustrate the distribution more clearly by showing the number of analyses falling into each particular group. Owing to the slight difference existing between the concentration of urea in the corpuscles and the plasma the " laked " and " unlaked " figures have been grouped together :—

From	3–4	mgm. N %	4	From	12–13	mgm. N %	0
	4–5	,,	7		13–14	,,	2
	5–6	,,	5		14–15	,,	2
	6–7	,,	4		15–16	,,	0
	7–8	,,	3		16–17	,,	0
	8–9	,,	1		17–18	,,	3
	9–10	,,	1		18–19	,,	3
	10–11	,,	2		19–20	,,	0
	11–12	,,	0		20–21	,,	2

From the above table it is evident that the majority of figures from " urea N " lie below 10 mgm. N per cent., viz., 25 out of 39, i.e., 64 per cent. It is noticeable that the first bloods done are mostly fairly high and, in my opinion, cannot be regarded as absolutely normal. Hamersma, in the work referred to earlier, dealing with hundreds of analyses, has found extraordinarily low values, less than 1·5 mgm. N per cent. The normal range lies from 2–10 mgm. N per cent., with the average at 4–7 mgm. N per cent. I would here also refer to the " normals " given in connexion with bluetongue (*vide* page 316), where the above findings are further substantiated.

Since the " urea N " in case of " laked " and " unlaked " filtrates is much the same, the same averages as given above apply. The " unlaked " filtrate, as a general rule, contains slightly less " urea N," the difference being, however, small—mostly less than 0·3 mgm. N per cent. The position with sheep is, as regards distribution of urea between plasma and cellular elements, thus similar to that recorded by Folin in human blood.

" *Total Creatinine* " *Nitrogen.*—This fraction represents the creatine N and the creatinine N, the creatine having been converted first into creatinine and the latter plus the preformed creatinine determined.

The variations in "laked" filtrates range from 2·08–2·66 mgm. N per cent. (5·6–7·2 mgm. creatinine) and in one case only exceeds the maximum given here, viz., 3·42 mgm. N per cent. Approximately half the cases lie between 2·08–2·15 mgm. N per cent. Although the variation is somewhat large in different sheep, figures for each individual are fairly constant, e.g., S. 32297 (Table 6), the amounts are 2·23, 2·61, 2·23, 2·42 mgm. N per cent. respectively, and in S. 29661 (Table 9) 2·13, 2·13 and 2·31 mgm. N per cent. respectively.

For "unlaked" filtrates the corresponding figures range from 1·4–2·15 mgm. N per cent. (4·0–5·8 mgm. creatinine) being only exceeded in two cases with 2·23 and 2·35 mgm. N per cent. The variation is, therefore, greater than is encountered with in "laked" bloods, although the amount of "total, creatinine" N is lower.

Amino-Acid Nitrogen.—This varies in the case of "laked" blood filtrate from 5·09–8·00 mgm. N per cent. (exceeded once only with 9·72) in the majority of cases falling into the 5·30–6·80 mgm. N per cent. group. For "unlaked" filtrates the range lies from 3·60–5·96 mgm. N per cent., with 4·67–5·64 mgm N per cent. being the largest group.

Uric Acid Nitrogen.—In the "laked" filtrate the normal variation is from 0·18–0·28 mgm. N per cent. (0·54–0·84 mgm. uric acid) with a narrower range of 0·18–0·23 mgm. N per cent. (0·54–0·69 mgm. uric acid). In the case of unlaked filtrates, determinations were always made, but the colour obtained was rather faint for accurate colorimetric readings when the dilutions were made up to 25 c.c. Subsequently a modification was introduced by diluting only to 15 c.c. when readings could be more accurately taken—a procedure that was followed in all later studies. The "normals" for uric acid will, therefore, be discussed under "Bluetongue" (*vide* page 316).

Non-Protein Nitrogen.—Great variations were noted here, the data ranging from 13·76–30·28 mgm. N per cent. for "laked" and 10·66–27·70 mgm. N per cent. for "unlaked" filtrates. The following two tables more clearly indicate this distribution and the groups in which most of the "normals" are concentrated.

(a) *Table for "laked" filtrates.*

From	13–14	mgm. N %	1
	14–15	,,	1
	15–16	,,	0
	16–17	,,	2
	17–18	,,	0
	18–19	,,	4
	19–20	,,	1
	20–21	,,	1
	21–22	,,	3
	22–23	,,	1
	23–27	,,	0
	27–28	,,	1
	28–29	,,	1
	29–30	,,	1
	30–31	,,	2

(b) *Table for "unlaked" filtrates.*

From	10–11	mgm. N %	1
	11–12	,,	1
	12–13	,,	2
	13–14	,,	3
	14–15	,,	3
	15–16	,,	3
	16–17	,,	1
	17–18	,,	0
	18–19	,,	1
	19–20	,,	1
	20–23	,,	0
	23–24	,,	1
	24–25	,,	1
	25–26	,,	0
	26–27	,,	1
	27–28	,,	1

The largest number of analytical figures lie from 10–17 mgm. N per cent. The higher N.P.N. figures I am inclined to hold to be really outside the true normal range, but no reason can at the moment be offered in explanation. The sheep appeared clinically healthy. A larger mass of data is required before valid "normals" can be laid down. These, for South African conditions, will soon be published by Hamersma and in connexion with the researches into bluetongue, further figures are supplied.

Heartwater Data.

Temperature Charts.—In order to indicate the type of temperature reaction, and more particularly to demonstrate at what periods of the reaction blood examinations have been made, charts have been incorporated. The periods at which blood has been drawn has been indicated on the curves by small circles. Temperatures were taken twice daily (once daily on Sundays) at 6.30 a.m. and 3.30–4 p.m., respectively. Only the actual reactions are recorded, the normal temperature records being omitted for the sake of economy of space, no useful purpose being served by the incorporation of several weeks of such normal records.

Tables of Data.—These are mostly self-explanatory, all constitutents being expressed in "mgm. per 100 c.c. of blood," except haemoglobin and total nitrogen, both of which are expressed as "grams per 100 c.c." The "coaguable nitrogen" has been obtained by calculation (Total N—N.P.N.). The "Rest nitrogen" represents the nitrogen fraction unaccounted for in any specific form after the "urea N" "Total creatinine N," "uric acid N" and "Amino acid N" had been subtracted from the "Non-protein N" figure. The urea (46·66 per cent. N), total creatinine (37 per cent. N) and uric acid (33·33 per cent. N) have for the sake of convenience been expressed both as such and as "Nitrogen." In the column "plasma" the symbols "n.u." (nothing unusual) refer to the physical appearance of the plasma, more particularly to its colour. In anaplasmosis, redwater and horsesickness, icteric plasmas were quite frequently encountered. The "plasma" column has been omitted in the case of heartwater, since the plasma at no time showed any haemolytic or icteric discoloration.

In the column "Temperature Reaction" the following symbols have been adopted :—

N (Normal)—meaning that no abnormal temperature reaction is going on at the time of bleeding.

P.I.N. (Post infectionem, normal)—indicating that the animal has been injected with virus, but that as yet no temperature reaction has set in.

R (Reaction)—indicating that blood was withdrawn during the course of a reaction.

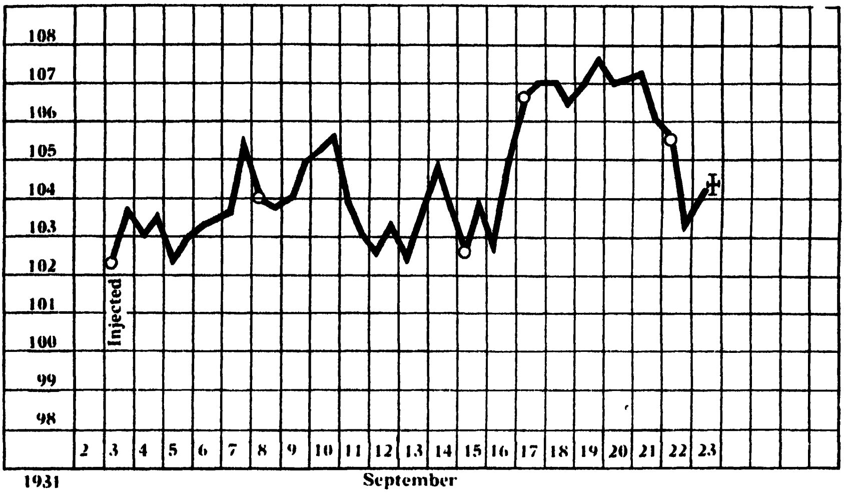
"Time of bleeding"—where no symbol is given it means that the blood was drawn between 8.30 a.m. and 9.30 a.m. In other cases the time of bleeding is inserted.

The above system has been applied throughout these publications.

HEARTWATER (*Rickettsia ruminantium* infection).

CASE I.

S. 27088: Killed *in extremis*, 23/9/31.



History.—Sheep 27088, hamel, six-tooth, carrying 1½ ins. wool, in good condition, weighing 109 lb. on 15/9/31. Passed through bluetongue in July, 1931. Was placed on temperature 2/9/31, and was injected intrajugularly on 3/9/31 with 10 c.c. mixed blood from heartwater sheep S. 32189 and S 31874. There was a temperature reaction from 4th to 8th day p.i. but there was no reflection of this slight reaction in the composition of the blood as examined on 8/9/31. The heartwater reaction began on 13th day p.i., the temperature rising within 36 hours to 107°, remaining at this level for four days and then dropping by crisis to 103·2°, the animal being killed *in extremis* on 20th day p.i. At the height of the reaction blood was injected into S. 31045 and S. 31761 (*vide*).

At the post-mortem examination (P.M. No. 10586, 23/9/31) the following pathological changes were found: slight hydrothorax and hydropericard, subendocardial haemorrhages, oedema of the lungs, tumor splenis, slight catarrhal enteritis, Preisz-nocard abscess in lung, fat necrosis, few oesophagostome nodules in caudal part of small intestines.

TABLE I.

S. 27088.							
Date.............		31/8/31.	3/9/31.	8/9/31.	15/9/31.	17/9/31.	22/9/31.
Time.............		—	—	—	—	—	—
Temp. R.........		N	N	P.I.N.	R	R	R
Hb. gm. %.......		13·79	13·12	13·31	12·13	11·78	11·67
Sugar mgm. %	L	56·80	39·53	51·00	77·70	93·10	50·00
	U	46·30	34·01	—	60·70	40 ?	28·6
T.N. gm. %.......		2·940	2·774	2·674	2·506	2·294	2·611
N.P.N. mgm. %	L	16·08	14·60	14·75	15·15	18·07	26·55
	U	12·62	11·75	—	11·70	12·50	20·54
Coag. N. gm. N %	L	2·924	2·759	2·659	2·491	2·276	2·584
	U	2·927	2·761	—	2·494	2·282	2·590
Urea mgm. %...	L	4·20 8·82	4·23 8·82	5·13 10·71	— —	— —	— —
	U	3·80 7·98	4·18 8·82	— —	— —	— —	— —
Total creatinine mgm. N %	L	— —	— —	— —	2·66 7·20	2·23 6·00	1·90 5·12
	U	— —	— —	— —	1·78 4·80	2·23 6·00	2·08 5·60
Uric Acid mgm. %	L	0·19 0·57	0·23 0·70	0·23 0·68	0·21 0·62	0·21 0·62	0·17 0·51
	U	TL TL	TL TL	TL TL	TL TL	TL TL	TL TL
Amino-acid mgm. %	L	8	6·28	5·13	5·76	6·19	5·56
	U	4·68	4·88	—	4·38	4·38	4·81
R.N. mgm. N %	L	4·68*	3·86*	5·26*	7·52†	10·34†	19·92†
	U	3·14‡	2·69‡	—	6·54§	6·89§	14·65§

* Includes "Total creatinine N."
† " "Urea N."
‡ " "Total creatinine and uric acid N."
§ " "Urea N" and "Uric acid N."

Salient Features Emerging from Analytical Data.

Hb.—A definite and steady drop from 13·79 gm. per cent. to 11·67 gm. per cent.

Sugar.—"Laked" filtrate shows a marked rise (51–93 mgm. N per cent.) corresponding to set in of the temperature reaction, followed by a drop to initial level on day preceding death. *In the "unlaked" the reverse occurs.*

T.N.—Shows a decrease succeeded by a rise towards end.

N.P.N.—Both "laked" and "unlaked" filtrates show a steady rise from 15·15–26·55 mgm. N per cent. and 11·70–20·54 mgm. N per cent. respectively.

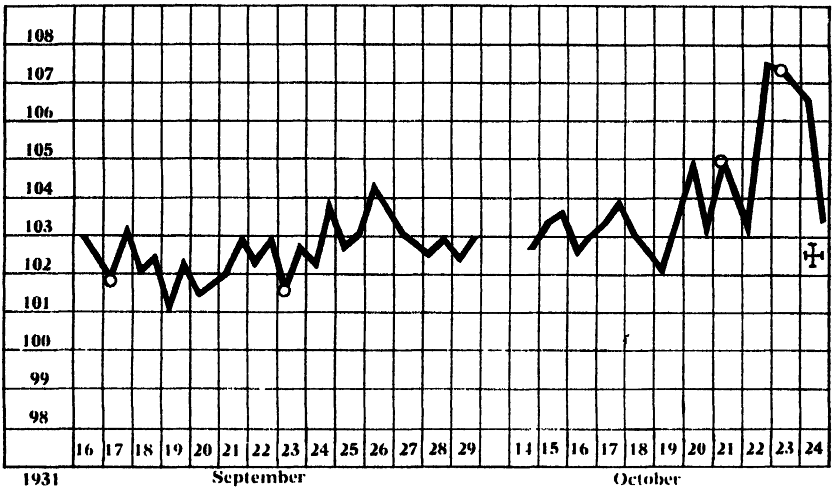
T.C.N.—Data incomplete, but in the case of "laked" filtrate there is a definite decrease (2·66–1·90 mgm. N per cent.). In the "unlaked" filtrate an initial rise is succeeded by a decrease on the day prior to death. The variations are within the normal range and may be of no special significance.

U.A.N.—The variations are small and within the experimental error and normal variation, but there seems a tendency towards a decrease as the disease progresses.

A.A.N.—The variations are within the normal range and no clear increase or decrease can be detected.

Case II.

S. 26689: Died 24/10/31.



History.—Sheep 26689, hamel, full mouth, carrying 1½ ins. wool, good condition, weighing 84 ℔. on 15/9/31 and 87 ℔. on 6/10/31. Passed through bluetongue in July, 1931. Was placed in heartwater experiment on 2/9/31 and injected on 3/9/31 intrajugularly with 10 c.c. blood from S. 32179 and S. 31874. After 17 days p.i. there was a slight temperature reaction, the maximum of 104·4° being reached on the 22nd day p.i., dropping to below 103° 48 hours later. This could not be regarded as a typical temperature reaction for heartwater in sheep or at the most as only a very mild reaction. It is, however, of interest to note that a blood examination undertaken on 23/9/31 just prior to the rise in temperature shows a marked rise in the "Urea N" content, viz.: 20·60 mgm. per cent. and 19·40 mgm. per cent. respectively for laked and unlaked filtrates. If this is compared with subsequent analyses taken during a later reaction, the suggestion is permissive, that the reaction is possibly a mild heartwater reaction. On the other hand, it should be noted that, if this reaction was due to heartwater, it conferred no immunity since exactly one month later (23/10/31) this animal succumbed to heartwater.

On 12/10/31 this sheep was again injected intrajugularly with 10 c.c. blood from S. 31109 (*vide*). On the 6th day p.i. the temperature reaction set in, the peak of 107·6° being reached four days later, the temperature falling during the next 48 hours by crisis to 104°, the animal dying on the 12th day p.i.

On post-mortem examination (P.M. No. 10548 of 25/10/31) there was found to be present marked hydrothorax, severe hydropericard, cyanosis of mucous membranes, oedema and hyperaemia of the lungs, subepicardial and subendocardial haemorrhages and slight catarrhal gastro-enteritis. The post-mortem findings, therefore, supported the diagnosis of heartwater.

TABLE II.

S. 26689.										
Date *Time*		31/8/31.	3/9/31.	8/9/31.	15/9/31.	17/9/31.	23/9/31.	13/10/31.	21/10/31.	23/10/31.
Temperature Reactions		N	N	P.I.N.	P.I.N.	R	R	P.I.N.	R	R
Haemoglobin gm. %		10·35	9·50	10·27	11·39	11·47	11·53	10·76	10·24	10·99
Sugar mgm. %	L	57·50	51·81	58·92	60·45	35·00	46·70	53·19	50·00	79·00
	U	50·00	38·46	—	37·00	30·00	37·40	35·80	48·54	64·90
Total N		2·373	2·401	2·373	2·429	2·429	2·590	2·527	2·569	2·590
N.P.N. mgm. %	L	17·80	13·76	21·13	21·90	19·36	35·30	16·48	23·62	35·30
	U	12·55	10·60	—	14·92	14·63	32·60	12·50	20·68	28·56
Coag. N. gm. N %	L	2·355	2·387	2·352	2·407	2·410	2·555	2·510	2·545	2·555
	U	2·360	2·390	—	2·414	2·414	2·557	2·515	2·548	2·561
Urea mgm. N %	L	4·70 9·87	3·42 7·40	— —	— —	— —	21·60 45·36	4·12 8·61	11·46 24·15	21·68 45·57
	U	3·50 7·35	3·40 7·14 *	— —	— —	— —	19·40 40·74	3·70 7·56	11·42 23·94	21·40 44·94
Total Creatinine mgm. N %	L	— —	— —	2·54 6·86	2·54 6·86	2·87 7·74	2·34 6·36	1·91 5·14	2·06 5·54	2·31 6·26
	U	— —	— —	— —	1·98 5·32	2·66 7·20	2·34 6·36	1·87 4·88	1·95 5·24	1·91 5·14
Uric acid mgm. N %	L	— —	0·27 0·81	0·28 0·84	0·32 0·96	0·23 0·78	0·21 0·63	0·21 0·62	0·66 2·00	0·28 0·83
	U	— —	TL TL	TL TL	TL TL	TL TL	TL TL	TL TL	0·19 0·58	TL TL
Amino-acid mgm. N %	L	7·30	6·80	6·22	7·18	7·45	7·48	7·29	6·67	5·18
	U	5·10	4·70	—	4·56	4·97	5·87	5·53	5·50	3·50
R.N. mgm. N %	L	6·80*	3·27†	13·09‡	12·86‡	9·81‡	4·48	2·73	2·77	5·85
	U	3·95*	2·50*	—	8·38§	8·00§	5·68‖	1·19‖	1·62	1·75

* Includes "Total creatinine N" and "Uric acid N." † Includes "Total creatinine N." ‡ Includes "Urea N."
§ Includes "Uric acid N" and "Urea N." ‖ Includes "Uric acid N."

Salient Features Emerging from Analytical Data.

Hb.—Nothing unusual.

Sugar.—During the initial slight reaction (nature uncertain) the level is very, low gradually rising thereafter in both "laked" and "unlaked" filtrates, reaching a level of 79 and 65 mgm. respectively on day before death.

T.N.—A tendency towards an increase exists, but the level remains within the normal limits. The increase is too small to permit of attaching any particular significance to it.

N.P.N.—Both filtrates show an increased nitrogen content during both the initial mild hyperthermic reaction and the second severe reaction (heartwater), with a return to normal in the interval between the two reactions.

U.N.—Similar to the N.P.N. curve, but much more marked, e.g., a rise from ± 4 mgm. N per cent. to 21·60 mgm. N per cent., with a drop to the initial level and a further marked increase during the second reaction.

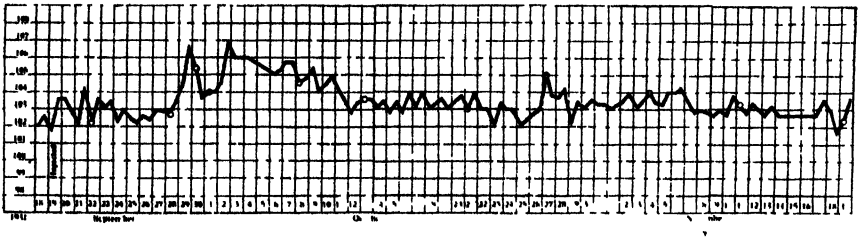
T.C.N. No very definite changes, but with a tendency towards an increase with the rise in temperature.

U.A.N.—Nothing definite, although a high U.A.N. content was encountered three days before death.

A.A.N. Nothing usual.

CASE III.

S. 31045: Recovered.



History.—Sheep 31045, two-tooth hamel, carrying 2½ ins. wool, fair condition, weighing 77 ℔. on 15/9/31 and 80 ℔. on 6/10/31. Passed through bluetongue in May, 1931. It was placed on temperature on 2/9/31 and was injected with 10 c.c. blood from S. 27088 (*vide*) on 19/10/31. The temperature reaction set in on the 10th day, reaching 106·8° on the 11th day, decreasing by lysis during the next twelve days to normal, i.e., the 22nd day p.i. The temperature remained normal, and on 1/12/31 an immunity test was performed, no reaction resulting.

TABLE III.

S. 31045. *Date* *Time*		22/9/31. —	28/9/31. —	30/9/31. —	1/10/31. —	8/10/31. —	13/10/31. —	21/10/31. —	27/10/31. —	4/11/31. —	11/11/31. —	19/11/31. —	1/12/31. —
Temperature Reactions		P.I.N.	P.I.N.	R	R	R	N	N	R	S.R.	N	N	N
Haemoglobin gm. %		10·56	9·94	10·14	10·14	9·32	8·49	9·81	9·81	8·49	8·49	10·14	10·99
Sugar mgm. %	L	—	43·48	73·56	55·55	29·70	52·63	47·62	60·00	36·00	50·25	42·55	42·37
	U	—	40·00	58·12	44·44	25·00	45·45	35·71	55·55	26·22	50·00	35·71	38·30
T.N. gm. N %		2·500	2·392	2·376	2·416	2·192	2·262	2·080	2·318	2·059	2·080	2·318	2·584
N.P.N. mgm. %	L	20·70	18·07	19·73	26·20	29·36	19·23	18·18	28·82	30·30	20·42	27·90	18·75
	U	16·30	15·46	15·39	21·90	24·06	15·85	14·28	21·82	25·20	15·00	21·43	13·50
Coag. N. gm. N %	L	2·479	2·374	2·356	2·390	2·163	2·243	2·062	2·289	2·029	2·060	2·290	2·565
	U	2·484	2·377	2·361	2·394	2·168	2·246	2·066	2·296	2·034	2·065	2·297	2·570
Urea mgm. N %	L	7·32	5·73	7·03	10·63	17·61	7·82	7·26	13·14	18·80	7·00	11·79	4·92
		15·33	11·97	14·70	22·26	36·96	16·38	15·33	27·51	39·48	14·70	24·78	10·29
	U	7·00	5·55	7·13	10·83	17·25	7·76	6·17	13·73	18·70	6·14	11·25	5·13
		14·70	11·76	14·91	22·68	36·33	16·38	13·02	28·77	39·27	12·81	23·73	10·71
Total Creatinine mgm. N %	L	2·08	2·31	1·91	2·31	2·13	1·98	1·91	1·78	2·17	2·42	2·42	2·31
		5·60	6·36	5·14	6·26	5·76	5·32	5·14	4·80	5·86	6·54	6·54	6·26
	U	2·06	2·01	1·78	1·98	1·82	1·82	1·82	1·82	1·78	2·13	2·23	2·17
		5·54	5·40	4·80	5·32	4·90	4·90	4·90	4·90	4·80	5·76	6·00	5·86
Uric acid mgm. N %	L	—	—	—	—	—	0·26	0·20	0·20	0·18	0·17	0·15	0·24
		—	—	—	—	—	0·79	0·61	0·60	0·54	0·51	0·44	0·73
	U	—	—	—	—	—	TL	0·13	TL	TL	TL	TL	0·14
		—	—	—	—	—	TL	0·40	TL	TL	TL	TL	0·43
Amino-acid mgm. N %	L	6·33	6·54	6·54	7·91	5·96	7·87	6·60	7·57	5·83	6·09	7·00	6·09
	U	4·73	5·76	4·46	5·67	3·84	5·87	5·09	5·38	3·91	4·75	6·36	4·24
R.N. mgm. N %	L	5·07*	3·45*	4·25*	5·35*	3·66*	1·30	2·20	6·13	3·32	4·72	6·54	5·19
	U	2·51*	2·14*	2·02*	3·42*	1·35*	0·46*	1·07	1·89*	0·81*	1·98*	1·59*	0·82

* Includes "Uric acid N."

Salient Features Emerging from Analytical Data.

Hb.—Shows tendency towards a drop in the Hb level during the hyperrhexic state.

Sugar.—Rather variable, the highest content being encountered during the hyperrhexias with 73·56 and 60 mgm. per cent. respectively, following by a decrease beyond the average level.

T.N.—Shows a gradual decrease, the lowest points being reached after the reaction has already returned to normal.

N.P.N.- A definite increase coinciding with the two temperature reactions.

U.N.—Both filtrates show a definite increase coinciding with the increase in body temperature from an initial level of ± 7 mgm. N per cent. to 18 mgm. N per cent.

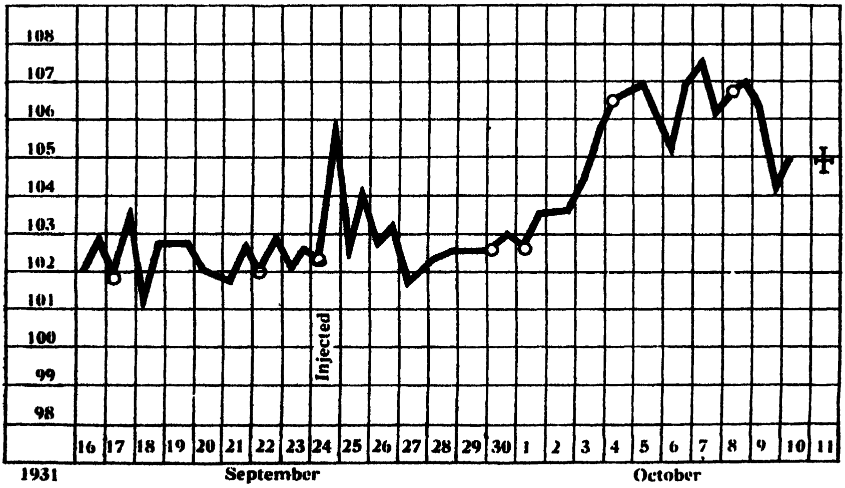
T.C.N.-- Variations cannot be definitely associated with the progress of the condition. With the second more severe hyperrhexia there is diminution of the "creatinine N" content, followed by a rise, but the differences encountered do not fall beyond the normal limits and may be coincidental.

U.A.N.—With the second reaction is associated a definite drop from 0·25 mgm. N per cent. to 0·15 mgm. N per cent. This decline is not directly dependent on body temperature, as it proceeds after the temperature has returned to normal.

A.A.N. --A decrease coinciding with the temperature reaction.

CASE IV.

S. 31028: Died 10/10/31.



History.—Sheep 31028, four-tooth hamel, carrying 1½ ins. wool 14/9/31, in fair condition, weighing 66 lb. on 15/9/31 and 67 lb. on 5/10/31. It passed through bluetongue reaction in April, 1931, and was placed in a bloedpens experiment during May, 1931, being drafted into the heartwater experiment on 2/9/31. It was injected with 10 c.c. blood mixture from S. 29651 and S. 28462 during the height of a heartwater reaction in these animals on 24/9/31. A severe heartwater reaction set in on the 9th day p.i., the

TABLE IV.

S. 31028.								
Date		17/9/31.	22/9/31.	24/9/31.	30/9/31.	1/10/31.	4/10/31.	8/10/31.
Time		—	—	—	—	—	—	—
Temperature Reactions		N	N	N	P.I.N.	P.I.N.	R	R
Haemoglobin gm. %		11·59	10·56	9·73	9·12	9·05	9·10	9·32
Sugar mgm. %	L	51·13	38·20	44·60	57·47	57·02	62·43	50·00
	U	38·84	26·20	31·20	57·14	41·60	48·00	39·20
T.N. gm. %		2·410	2·442	2·330	2·290	2·332	2·310	2·339
N.P.N. mgm. %	L	22·90	22·10	35·30	26·09	20·00	48·89	63·84
	U	15·70	14·92	29·30	22·90	15·36	43·36	53·58
Coag. N gm. N %	L	2·387	2·419	2·295	2·254	2·312	2·263	2·275
	U	2·394	2·427	2·301	2·257	2·317	2·269	2·285
Urea mgm. N %	L	—	—	—	13·71	7·73	32·06	42·18
„ U %	U	—	—	—	28·77	16·17	67·41	88·62
„ N %	L	—	—	—	13·00	7·55	31·83	37·37
„ U %	U	—	—	—	27·30	15·96	66·78	78·54
T.C. mgm. N %	L	2·40	3·42	2·66	2·13	2·06	1·98	2·54
„ TC %		6·48	6·54	7·20	5·76	5·54	5·32	6·86
„ N %	U	1·98	2·23	1·67	2·06	1·75	1·67	2·17
„ TC %		5·32	6·00	4·50	5·54	4·70	4·50	5·84
Uric Acid mgm. N %	L	0·18	—	0·21	—	—	—	—
„ UA %		0·53	—	0·62	—	—	—	—
„ N %	U	TL	TL	TL	TL	TL	TL	—
„ UA %		TL	TL	TL	TL	TL	TL	—
Amino Acid mgm. N %	L	5·83	6·39	5·74	7·37	6·54	6·39	5·56
	U	5·13	5·28	5·00	4·98	4·32	5·20	4·12
R.N. mgm. N %	L	15·49*	13·29†	20·69*	2·88‡	3·67‡	8·46‡	13·56‡
	U	9·59†	7·40†	22·63†	2·86‡	1·74‡	4·66‡	11·92‡

* Includes "Urea N." † Includes "Urea N" and "Uric acid N." ‡ Includes "Uric acid N."

temperature maximum being 107·6° dropping by crisis on 15th day p.i., when the animal died. The post-mortem findings (P.M. No. 10624 of 11/10/31) supported the diagnosis of heartwater, the chief pathological findings being cyanosis of the mucous membranes, slight hydrothorax, hydropericard, hydroperitoneum, subendocardial and subepicardial haemorrhages and oedema of the lungs, fatty degeneration of the liver, catarrhal haemorrhagic gastritis, few oesophagostome nodules in the latter portion of the small intestines.

Salient Features emerging from Analytical Data.

Hb.—A drop in this is noted, but this would not appear to be associated with the pathological condition since the decrease had already set in before even the virus was injected. On the contrary, there are indications of a slight increase during the hyperrhexic state.

Sugar.—Shows an increase level during reaction succeeded by a fall shortly before death.

T.N.—Slight decrease, possibly related to Hb. content.

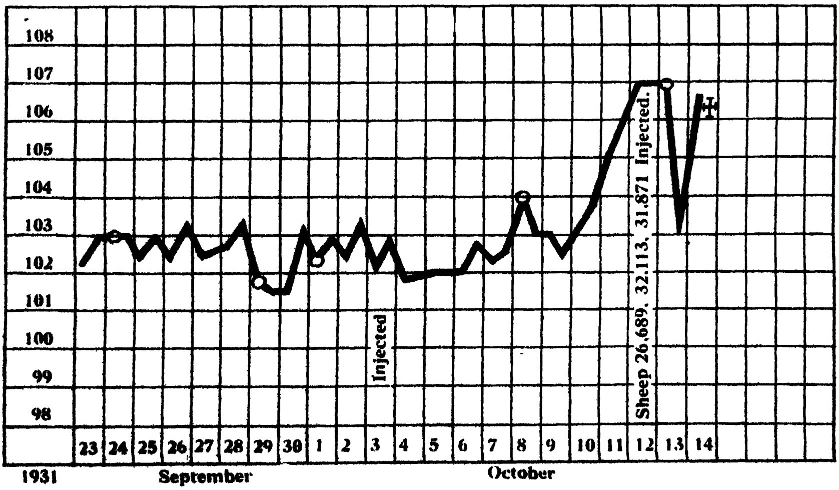
N.P.N. and U.N.—Show a definite and marked rise in both filtrates, the N.P.N. up to 63·84 mgm. N per cent. and 53·58 mgm. N per cent. respectively; the U.N. from about 7 mgm. N per cent. to 32 mgm. N per cent.

T.C.N.—This animal shows initially a high level for T.C.N., but during the reaction there is a distinct if slight drop, with again a higher T.C.N. shortly before death. This applies to both filtrates.

R.N.—Although the U.A.N. is included there is a marked increase towards the end of reaction.

CASE V.

S. 31109: Died 14/10/31.



History.—Sheep 31109, four tooth hamel, carrying 2 ins. wool, good condition, weighing 87 ℔. on 15/9/31 and 96 ℔. on 6/10/31. Passed through bluetongue in April, 1931, and placed in heartwater experiment on 2/9/31. Was injected on 3/10/31 with 5 c.c. blood intrajugularly from heartwater sheep S. 31761 (*vide*) and S. 31426. On the 6th day p.i. the temperature reaction set in, the temperature steadily rising over three days to 107°, dropping by crisis to 103° within 24 hours, the temperature rising by next morning again to 106·8°, the animal dying shortly after on the 11th day p.i. At the height of the reaction blood was injected from this sheep into S. 26689 and S. 32113 and S. 31871 (*vide*).

At the post-mortem examination (P.M. No. 10630 of 15/10/31) the pathological changes consisted of hydropericard, hydroperitoneum, hydrothorax, hyperaemia and oedema of the lungs, and slight tumor splenis.

TABLE V.

S. 31109.					
Date *Time*	24/9/31. —	29/9/31. —	1/10/31. —	8/10/31. —	13/10/31. —
Temp. React	N	N	N	P.I.N.	R
Hb. gm. %	12·01	12·42	11·80	14·95	9·73
Sugar mgm. % L	32·90	48·78	—	51·00	53·19
U	26·30	31·16	—	34·10	36·60
T.N. gm. %	2·680	2·710	2·521	2·934	2·388
N.P.N. mgm. % L	34·50	36·60	28·58	36·36	27·78
U	31·60	34·60	22·00	29·00	25·27
Coag. N gm. N % L	—	2·673	2·492	2·898	2·359
U	—	2·675	2·499	2·905	2·363
Urea mgm. N % L	20·45 43·05	18·46 38·85	— —	20·86 43·89	17·34 36·33
U	20·02 42·00	18·60 39·06	— —	20·86 43·89	17·06 35·91
Total Creatinine mgm. N % L	2·10 5·68	2·13 5·76	— —	2·31 6·26	2·06 5·54
U	2·15 5·80	1·91 5·14	— —	1·98 5·32	1·98 5·32
Uric Acid mgm. N % L	0·24 0·72	0·20 0·59	0·17 0·50	0·21 0·63	0·30 0·89
U	TL TL	TL TL	TL TL	TL TL	TL TL
Amino Acid mgm. N % L	7·00	7·33	5·88	7·07	6·97
U	5·58	4·62	—	4·75	4·95
R.N. mgm. N % L	4·71	8·48	—	5·91	2·11
U	4·29*	9·47*	—	1·48*	1·28*

* Includes "Uric acid N."

Salient Features Emerging from Analytical Data.

Hb.—Very irregular, lowest content on day prior to death.

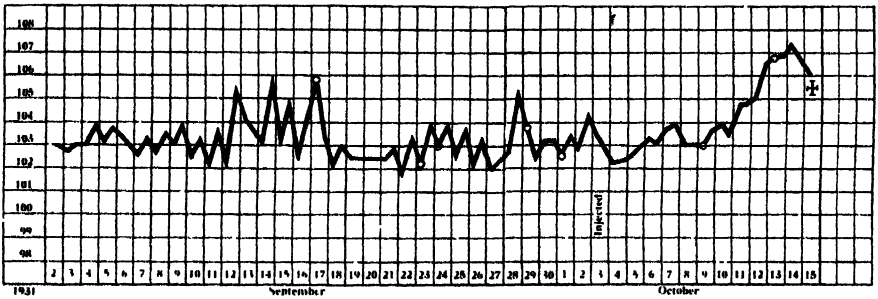
Sugar.—A tendency towards an increase.

N.P.N. and *U.N.*—It will be noted that both these N fractions of the blood remained consistently high over the entire period over which this animal was examined. No explanation can at present be offered since neither a clinical examination nor the post-mortem findings suggest any specific cause.

CASE VI.

S. 32297: Died 15/10/31.

Temperature Chart VI.



History.—Sheep 32297, two-tooth hamel, carrying 1 in. wool, condition fair, weighing 55 lb. 15/9/31 and 70 lb. 6/10/31. Suffering from ophthalmia of right eye on 14/9/31. Passed through bluetongue in May, 1931. Placed into heartwater experiment on 2/9/31 and injected with 5 c.c. blood intrajugularly from heartwater sheep 31761 (*vide*) and S. 31426 on 3/10/31. From the 8th day p.i. the temperature began to rise till the 11th day, reaching 107·2°, dropping during the next day to 106°, the animal dying in the late afternoon, i.e., on the 12th day p.i.

On post-mortem examination (P.M. No. 10632 of 16/10/31) there was found to be present hydropericardium, slight oedema and hyperaemia of the lungs, slight tumor splenis and impaction of the oesophagus. No worms were found in the stomach and intestines. The condition of the carcass was good. The post-mortem findings, therefore, support the diagnosis of heartwater.

TABLE VI.

S. 32297.							
Date.............		17/9/31.	23/9/31.	29/9/31.	1/10/31.	9/10/31.	13/10/31.
Time.............		—	—	—	—	—	—
Temp. R.........		R	R	R	N	P.I.N.	R
Hb. gm. %.......		11·59	10·56	11·59	11·59	11·96	8·12
Sugar mgm. %	L	41·66	39·30	38·46	—	57·14	96·16
	U	31·00	34·40	33·78	—	42·01	44·44
T.N. gm. %.......		2·575	2·571	2·613	2·559	2·500	2·112
N.P.N. mgm. %	L	21·43	28·57	23·44	21·90	25·00	22·64
	U	19·60	26·10	—	13·67	22·31	20·98
Coag. N. gm. N %	L	2·554	2·542	2·590	2·537	2·475	2·099
	U	2·555	2·545	—	2·545	2·478	2·101
Urea mgm. N %	L	10·94	17·35	8·09	6·30	14·51	12·50
		22·89	36·54	17·01	13·23	30·45	26·25
	U	10·69	17·02	9·71	4·40	14·21	12·94
		22·47	35·70	20·37	9·24	29·82	27·09
~~Total Creatinine~~	L	2·23	2·61	2·23	2·42	2·04	2·23
mgm. No. %		6·00	7·06	6·00	6·54	5·50	6·00
	U	1·98	2·35	2·06	1·75	2·01	1·84
		5·32	6·36	5·54	4·70	5·40	4·96
Uric Acid	L	0·27	0·23	0·21	0·18	0·18	0·21
mgm, N %		0·80	0·68	0·62	0·53	0·53	0·64
	U	TL	TL	TL	TL	TL	TL
		TL	TL	TL	TL	TL	TL
Amino Acid mgm. N %	L	5·64	5·09	6·31	5·09	6·01	5·15
	U	4·97	4·67	4·49	3·60	4·93	4·20
R.N. mgm. N %	L	2·35	3·29	6·60	8·81	2·26	2·55
	U	1·96*	2·06*	—	0·88*	1·16*	2·00*

* Includes "Uric acid N."

Salient Features of Analytical Data.

Hb.—A distinct drop just prior to death.

Sugar.—Shows a marked rise to 96·16 mgm. per cent. and 44·44 mgm. per cent. in "laked" and "unlaked" filtrates respectively. In the "laked" the relative rise is more evident, and it is of interest to note that the increase in the case of the "unlaked" is not parallel to the former, the blood sugar being less in the latter by over a 100 per cent. This aspect will be discussed later under "General Discussion."

T.N.—A decrease is noted towards exitus lethalis.

N.P.N.—No definite variations, the level throughout being on the high side. For this condition a temperature reaction of unknown etiology during September is apparently responsible, it being reflected by the slightly increased N.P.N. during this period.

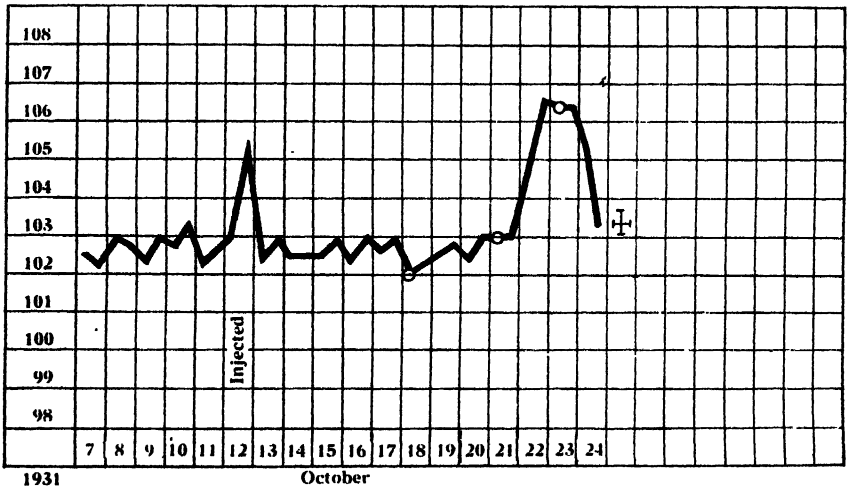
U.N.—The initial high U.N. is associated with the reaction referred to above, the normal level being again reached prior to the injection with virus, succeeded by a slight rise during the heartwater reaction.

U.A.N.—During the first temperature reaction this fraction is just above the upper limit of normal, gradually diminishing.

CASE VII.

S. 31871: Died 24/10/31.

Temperature Chart VII.



History.—Sheep 31871, hamel, four-tooth, carrying 1 in. wool on 14/9/31, fair condition, weighing 54 ℔. on 15/9/31 and 59·5 ℔. on 6/10/31. Passed through bluetongue reaction in April, 1931. Was placed into the heartwater experiment on 2/9/31 and injected intrajugularly on 12/10/31 with 10 c.c. blood from heartwater sheep 31109 (*vide*). On the 9th day p.i. the temperature reaction set in, rising within 24 hours to 106·8° and dropping during the next 48 hours to 103·2°, the animal dying on the 13th day p.i. On the day prior to death 10 c.c. blood was injected intrajugularly into sheep 29661 and sheep 31011 (*vide*). On post-mortem examination (P.M. No. 10649 of 26/10/31) the following pathological changes were noted: hydropericard, hydrothorax, cyanosis of mucous membranes, degeneration of myocard, subendocardial and subepicardial haemorrhages, slight tumor splenis and a slight oedema of the lungs. The carcass was in fair condition. The post-mortem findings, therefore, support the diagnosis of heartwater.

TABLE VII.

S. 31871.					
Date	18/9/31.	28/9/31.	1/10/31.	21/10/31.	23/10/31.
Time	—	—	—	—	—
Temp. R	N	N	N	R	R
Hb. gm. %	9·52	8·49	9·52	9·32	9·40
Sugar mgm. % L	73·00	52·63	50·00	60·00	68·50
U	68·50	45·45	30·00	48·00	70·43
T.N. gm. %	2·362	2·180	2·340	2·213	2·024
N.P.N. mgm. % L	19·67	30·90	16·82	22·23	40·54
U	14·51	27·70	13·49	21·63	35·30
Coag. N gm. N % L	2·342	2·149	2·323	2·191	1·983
U	2·347	2·152	2·327	2·191	1·991
Urea mgm. N % L	—	18·19	4·29	12·20	24·25
	—	38·22	9·03	25·41	51·03
U	—	17·84	4·47	11·40	23·92
	—	37·38	9·45	23·94	50·19
Total Creatinine mgm. N % L	2·15	2·66	2·31	2·23	2·23
	5·80	7·20	3·26	6·00	6·00
U	1·67	2·10	1·32	1·98	1·98
	4·50	5·68	4·90	5·32	5·32
Uric Acid mgm. N % L	0·22	0·22	0·22	0·37	0·23
	0·66	0·66	0·66	1·12	0·69
U	TL	TL	TL	0·21	0·15
	TL	TL	TL	0·64	0·45
Amino acid mgm. N % L	6·03	7·00	6·28	6·83	5·18
U	4·47	4·95	4·97	5·96	4·67
R.N. mgm. N % L	—	3·05	3·72	0·59	7·50
U	—	1·81*	2·23*	2·08	4·73

* Includes "Uric Acid N."

Salient Features of Analytical Data.

Sugar.—Shows an increase with rise in temperature.

T.N. N.P.N. and *U.N.*—On the 28th September a drop occurs, associated with a low Hb content and a relatively increased N.P.N. and U.N. not associated with any hyperthermia. During the heartwater reaction the T.N. and N.P.N. again diminishes, in this case unassociated with a decline in the Hb. level.

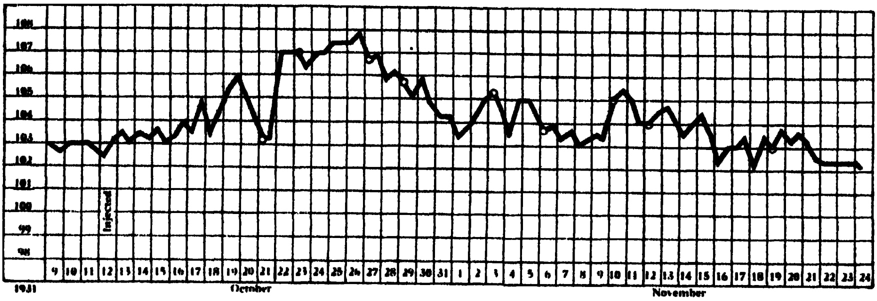
U.A.N.—With the onset of the reaction this is increased but rapidly drops to the normal level.

R.N.—This fraction is relatively high shortly before death.

CASE VIII.

S. 32113: Recovered.

Temperature Chart VIII.



History.—Sheep 32113. four-tooth hamel, carrying 1 in. wool on 14/9/31, in good condition, weighing 77 lb. on 14/9/31 and 80 lb. on 6/10/31. Passed through bluetongue in June, 1931. This animal was placed on temperature on 2/9/31. It was injected on 12/10/31 with 10 c.c. blood from S. 31109, which was suffering from heartwater. Six days later the temperature rose, reaching 106° on the 8th day, dropping to 103° on the 10th day, rising again on the 9th day to 107°, remaining at between 107° and 108° for six days and then decreasing by lysis to reach 103° in twelve days (27th day p.i.). On 1/12/31 it was again injected with 5 c.c. virulent heartwater blood, but no reaction occurred.

Salient Features of Analytical Data.

Temperature Reaction.—The hyperthermia encountered here is *atypical* for heartwater, although no doubt as to its origin exists. It is a severe long drawn out reaction followed by a milder, though distinct hyperthermia, during which the blood changes are similar to those encountered during the main reaction only to a somewhat lesser degree. It ended in ultimate recovery, but from a clinical point of view the issue was doubtful for several days, the animal being down for 72 hours. In my opinion the long drawn out temperature reaction is an expression of the strong resistance put up against the infection. This case, therefore, represents a more complete and accurate picture as to the changes involved in the composition of the blood during heartwater than is the case of a reaction ending abruptly in death.

Hb.—A well-marked decrease in the Hb. content (from 9·12–7·68 gm. per cent.) is noted, coinciding with the main reaction.

Sugar.—Shows an increase during the main reaction, preceded by a decrease at its onset.

T.N.—Runs parallel with the Hb. content, the decrease in T.N. coinciding with the drop in haemoglobin values.

N.P.N. and *U.N.*—Both these fractions show a marked rise during the hyperthermic reactions. The N.P.N. rises from about 19 mgm. N per cent. to 51·72 mgm. N per cent. in the "laked" filtrate, returning to normal and increasing again to 25·54 mgm. N per cent. during the lesser reaction. In the "unlaked" filtrate the same phenomenon is observed, only on the lower level characteristic for this type of filtrate. The U.N. content runs parallel to the N.P.N., rising from 6·5 mgm. N per cent. to 37·10 mgm. N per cent ("unlaked" slightly lower), then returning to normal, followed by a slight increase to a little over the maximum range of the normal variation.

TABLE VIII.

S. 32113.													
Date		28/9/31.	1/10/31.	21/10/31.	23/10/31.	27/10/31.	29/10/31.	3/11/31.	6/11/31.	10/11/31.	12/11/31.	19/11/31.	1/12/31.
Time		—	—	—	—	—	—	—	—	—	—	—	—
Temperature Reactions		N	N	R	R	R	R	R	N	R	R	R	N
Haemoglobin gm. %		8·90	9·94	9·12	8·53	7·47	7·68	9·05	8·07	9·32	8·80	9·81	8·65
Sugar mgm. %	L	66·92	53·20	38·00	60·61	62·89	53·48	71·43	48·58	57·47	58·14	42·01	38·46
	U	58·80	43·48	35·71	54·65	55·87	40·82	66·67	42·37	52·36	47·17	42·37	31·75
T.N. gm. %		2·310	2·560	2·351	2·192	2·010	2·097	2·136	2·080	2·255	2·248	2·374	2·136
N.P.N. mgm. %	L	27·90	18·63	21·45	26·08	40·82	44·51	51·72	19·73	20·00	25·54	23·08	15·95
	U	23·44	15·71	18·18	22·56	37·50	42·80	46·16	16·02	15·39	20·52	19·36	12·53
Coag. N gm. N %	L	2·282	2·541	2·330	2·166	1·969	1·952	2·084	2·060	2·235	2·222	2·351	2·120
	U	2·287	2·544	2·333	2·169	1·972	1·954	2·090	2·064	2·240	2·227	2·355	2·123
Urea mgm. N %	L	13·80 28·98	6·50 13·65	10·40 28·84	15·00 31·50	26·97 56·70	33·78 70·98	37·10 77·70	9·31 19·53	7·08 14·91	12·87 27·09	10·94 22·89	4·80 10·08
	U	13·80 28·98	6·40 13·44	10·95 23·10	14·33 30·03	26·03 54·60	34·10 71·61	35·04 73·50	8·95 18·90	7·36 15·54	12·56 26·46	10·77 22·68	4·13 8·61
Total Creatinine mgm. N %	L	2·10 5·68	2·13 5·76	2·17 5·86	2·06 5·54	1·84 4·96	1·98 5·32	3·26 8·80	2·27 6·16	— —	3·80 10·28	2·66 7·20	2·31 6·26
	U	1·98 5·32	1·82 4·90	1·98 5·32	1·91 5·14	1·54 4·16	1·91 5·14	2·23 6·00	2·11 5·68	— —	2·74 7·40	2·42 6·54	2·06 5·54
Uric acid mgm. N %	L	— —	0·24 0·71	0·23 0·69	0·22 0·66	0·23 0·67	0·27 0·80	0·30 0·89	0·17 0·50	0·22 0·66	0·29 0·87	0·21 0·62	0·23 0·70
	U	— —	TL TL	0·08 0·23	TL TL	TL TL	TL TL	TL TL	TL TL	TL TL	TL TL	TL TL	0·15 0·44
Amino-acid mgm. N %	L	5·30	6·28	5·60	5·60	5·00	4·44	6·51	5·83	5·60	7·18	6·67	6·36
	U	4·71	5·64	5·00	4·31	3·68	3·89	4·12	3·89	4·24	4·88	5·18	5·18
R.N. mgm. N %	L	6·70*	3·48	3·28	2·20	7·79	4·04	4·55	2·15	7·32†	1·46	2·58	2·25
	U	2·95*	1·85*	1·10	2·01*	4·25*	2·90*	4·77*	1·07*	3·79‡	0·34*	1·01*	1·00

* Includes "Uric acid N." † Includes "Total creatinine N." ‡ Includes "Total creatinine N" and "Uric acid N."

T.C.N.—In both filtrates there is a decrease, coinciding with the hyprethemia associated with the main heartwater reaction. Thereafter there is an increase reaching 3·60 mgm. N per cent. during the secondary reaction.

U.A.N.—Shows a tendency towards a rise during main reaction.

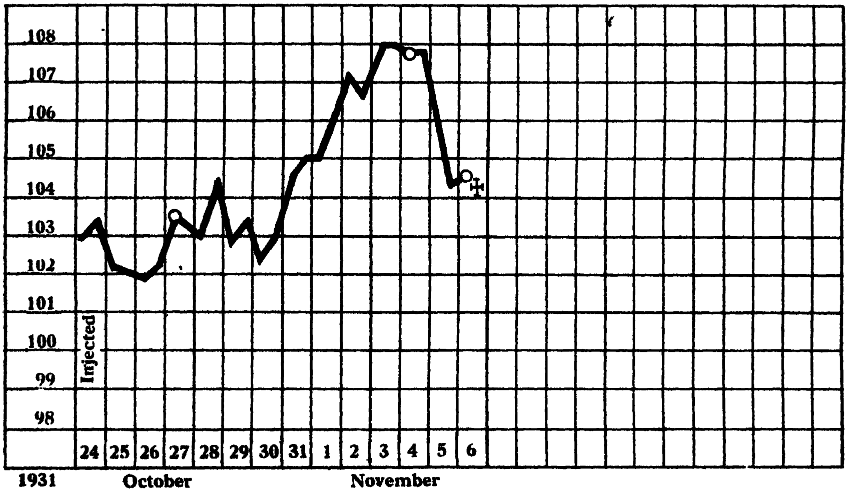
A.A.N.—Nothing unusual.

R.N.—During the initial reaction this [fraction increased in amount to approximately 8 mgm. N per cent. and 4 mgm. N per cent. in "laked" and "unlaked" filtrates respectively.

CASE IX.

S. 29661: Died 6/11/31.

Temperature Chart IX.



History.—Sheep 29661, six-tooth hamel, carrying 3¼ ins. wool 14/9/31, in good condition, weighing 77 lb. on 15/9/31 and 80 lb. on 6/10/31. Passed through bluetongue in July, 1931. Was placed on temperature 2/9/31 and was injected on 24/10/31 with 10 c.c. blood from sheep 31871 (*vide*) which was suffering from heartwater. The temperature reaction set in on the 7th day, the temperature steadily rising to reach 108° on the 4th day after the onset, remained at 108° for two days and then fell by crisis within 24 hours to 104°, the sheep dying on the 13th day p.i.

On post-mortem examination there was found to be a general cyanosis, slight hydrothorax and hydropericard, oedema of the lungs, fatty changes in the liver and the kidneys, tumor splenis, subendocardial and subepicardial haemorrhages, findings which support the diagnosis of heartwater.

TABLE IX.

S. 29661.						
Date..............	18/9/31.	30/9/31.	14/10/31.	27/10/31.	4/11/31.	6/11/31.
Time.............	—	—	—	—	—	—
Temp. React......	N	N	N	P.I.N.	R	R
Hb. gm. %........	10·97	11·18	10·97	11·67	10·14	12·42
Sugar mgm. %..L	62·11	45·54	50·00	53·96	55·87	156·24
U	50·60	42·54	34·40	45·15	45·66	151·50
T.N. gm. %.......	2·370	2·480	2·465	2 458	2·262	2·532
N.P.N. mgm. % L	22·22	30·28	18·79	20·00	40·54	31·74
U	18·52	24·00	14·77	16·27	36·48	25·00
Coag. N. L	2·348	2·430	2·446	2·438	2 221	2·500
gm. N % U	2·351	2·456	2 456	2·442	2·226	2·505
Urea mgm. N % L	—	14·40	5·89	6·87	29·06	22·00
	—	30·24	12·39	14·49	61·11	46·20
U	—	14·04	5·76	7·50	29·77	20·05
	—	29·40	12·18	15·75	62·68	42·21
Total Creatinine L	2·13	2·31	2·31	2·02	2·17	1·91
mgm. N %	5·76	6·26	6·26	5·42	5·86	5·14
U	1·67	1·49	1·49	1·54	1·75	1·37
	4·50	4·00	4·00	4·16	4·70	3·48
Uric Acid L	0·25	0·22	0·24	0·21	0·26	0·25
mgm. N % U	0·76	0·67	0·71	0·62	0·77	0·76
	—	—	—	—	—	0·18
	—	—	—	—	—	0·53
Amino acid L	6·73	9·72 ?	7·53	7·73	5·18	4·67
mgm. N % U	5·96	4·56	5·64	5·38	4·12	3·18
R.N. mgm. N % L	14·11*	4·63	2·39	3·17	3·87	2·91
U	12·16†	3·91‡	1·88‡	1·85‡	0·84‡	1·78

* Includes "Urea Nitrogen.
† „ "Uric acid N" and "Urea N."
‡ „ "Uric acid N."

Salient Features of Analytical Data.

Sugar.—This shows a gradual increase up to 56 mgm. per cent. with a very high level (156 mgm. per cent.) a few hours before death on 6/11/31.

N.P.N. and *U.N.*—Both evince the usual feature of an increase to 41 and 37 mgm. N per cent. respectively for N.P.N. in both filtrates succeeded by a decline on day of death. The U.N. curve is similar, being 29 mgm. N per cent. (61 mgm. urea).

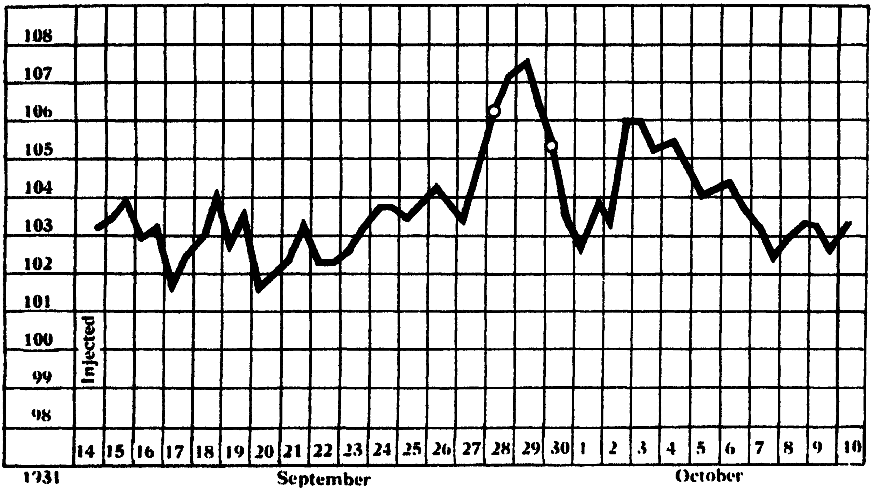
T.C.N.—Here a decline from 2·31 mgm. N per cent. to 1·91 mgm. N per cent. is noted.

A.A.N.—Shows a relatively low level a few hours before death.

Cases X, XI, and XII.

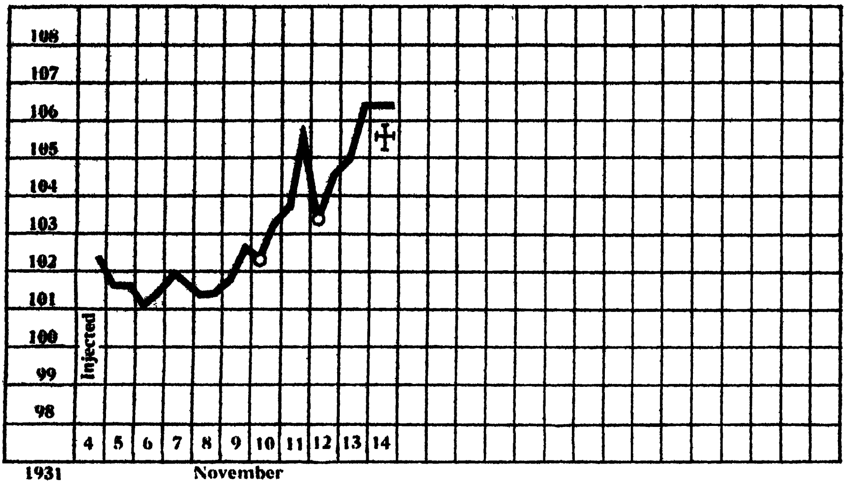
S. 31051 : Recovered.

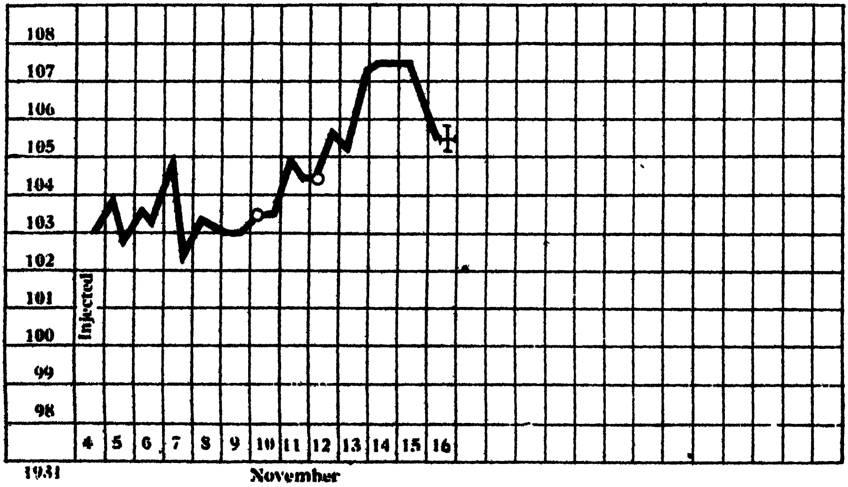
Temperature Chart X.



History.—Sheep 31051, four-tooth hamel, carrying 3 ins. wool, good condition, weighing 75 lb. on 15/9/31 and 74 lb. on 6/10/31. Passed through bluetongue in May, 1931. The animal was placed on temperature on 14/9/31 and injected on the same day with 20 c.c. of blood from a calf which had died of heartwater (natural infection). On the 13th day the temperature rose, reaching 107·6° on the 15th day, gradually subsiding to normal by the 24th day p.i.

Temperature Charts XI and XII. Sheep 31873 and 31144.





History.—Sheep 31873 and 31144. These sheep were injected with 10 c.c. blood each intrajugularly from S. 29661 (*vide*) on 4/11/31. Both temperature reactions set in on the 7th day, reaching 107·4° on the 10th day p.i., S. 31873 dying on the 11th day p.i. and S. 31144 on the 13th day at the height of the temperature reaction. Both were in good condition.

S. 31873 at the post-mortem examination (P.M. No. 10677 of 15/11/31) showed slight hydrothorax and hydropericard, subendocardial haemorrhages, hyperaemia and oedema of the lungs, ulcerative abomasitis and acute enteritis.

S. 31144 (P.M. No. 10678 of 16/11/31) showed anaemia, oedema of the lungs, hydrothorax, ascitis, subepicardial and subendocardial haemorrhages, nodular enteritis, tumor splenis and fatty changes in the liver and kidneys.

TABLE X.

S. 31051.				
Date		3/9/31.	28/9/31.	30/9/31.
Time		—	—	—
Temperature Reaction		N	R	R
Haemoglobin gm. %		9·12	8·90	9·13
Sugar mgm. %	L	—	60·00	46·51
	U	—	37·00	40·00
Total Nitrogen gm. N %	L	2·350	2·300	2·297
Non-Protein Nitrogen mgm. %	L	18·70	24·50	27·03
	U	13·00	22·30	20·98
Coaguable Nitrogen gm. N %	L	2·331	2·275	2·270
	U	2·338	2·278	2·276
Urea mgm. N %	L	—	13·37	12·77
		—	28·14	26·88
	U	—	13·04	12·99
		—	27·30	27·30
Total Creatinine mgm. N %	L	—	2·23	1·91
		—	6·00	5·14
	U	—	1·75	1·78
		—	4·70	4·80
Uric Acid mgm. N %	L	0·21	TL	TL
		0·63	TL	TL
	U	TL	TL	TL
		TL	TL	TL
Amino Acid mgm. N %	L	5·74	5·49	5·11
	U	4·67	5·11	3·99
Rest Nitrogen mgm. N %	L	—	3·41*	7·24*
	U	—	2·40*	2·22*

* Includes "Uric Acid N."

TABLE XI. TABLE XII.

S. 31873.				S. 31144.	
Date *Time*		10/11/31. —	12/11/31. —	10/11/31. —	12/11/31. —
Temperature Reactions		R	R	R	R
Haemoglobin gm. %		13·41	—	12·98	10·14
Sugar mgm. %	L	42·37	46·95	39·37	51·02
	U	33·56	38·46	38·46	42·37
T.N. gm. N %		2·710	2·570	2·696	2·528
N.P.N. mgm. %	L	19·08	30·00	16·94	23·06
	U	14·63	25·64	12·25	18·04
Coag. N. gm. N %	L	2·691	2·540	2·679	2·505
	U	2·695	2·546	2·684	2·510
Urea mgm. N %	L	7·00 14·70	17·00 35·70	4·28 9·03	9·10 19·11
	U	6·02 12·60	15·00 31·50	4·25 9·03	9·02 18·90
Total Creatinine mgm. N %	L	— —	2·66 7·20	— —	2·73 7·58
	U	— —	2·54 6·86	— —	2·48 6·70
Uric Acid mgm. N %	L	0·22 0·66	0·30 0·91	0·22 0·66	0·27 0·86
	U	TL TL	TL TL	TL TL	TL TL
Amino acid mgm. N %	L	6·36	6·93	6·51	7·57
	U	5·49	4·91	4·67	5·22
R.N. mgm. N %	L	5·48*	3·11	5·93*	3·39
	U	3·12†	3·19‡	3·33†	2·32‡

* Includes "Total Creatinine N."
† „ "Total Creatinine N" and "Uric Acid N."
‡ „ "Uric Acid N."

Salient Features of Analytical Data.

These three cases are grouped together owing to the small number of analyses available for each.

Hb.—In case of XII a decrease from 12·98 gm. per cent. to 10·42 gm. per cent. occurs.

N.P.N. and *U.N.*—All show slight increases, the relatively low levels when compared with other cases being probably due to the acute nature of the reactions.

T.C.N.—In Case X a decrease is noted; in the other cases no comparative figures are available.

GENERAL SUMMARY AND DISCUSSION.

Haemoglobin (Hb.).—The haemoglobin content during any particular period normally shows variations not only in the same animal from practically day to day, but also in different animals of the same species. Factors such as age, sex, condition, time of watering in relation to the period at which the blood is examined, all contribute towards a variable Hb. content. It follows, therefore, that a " mean " Hb. curve can only be arrived at if a number of successive determinations are undertaken. In such a case, although there may be variations, the " mean " level can readily be noted. In the present series of investigations care was taken to obviate the introduction of an anaemia due to too frequent bleedings of one and the same animal.

Under pathological conditions various circumstances may produce a change in Hb. content, generally in the direction of oligo-chromaemia, hyperchromaemia being less frequently encountered.

With regard to heartwater no very striking changes in the Hb. content as a sequel to infection emerge. An analysis of the cases presented show that (*a*) a tendency towards oligo-chromaemia is present in over 50 per cent. of cases (Cases I, III, IV, V, VI, VIII and XII). In some cases the decrease is direct, e.g. in Case I from 13·79 gm. per cent. to 11·67 gm. per cent.: in Case VIII from 9·13 gm. per cent. to 7·68 gm. per cent.; in others an initial drop is succeeded by an increase usually towards the termination of the temperature reaction or shortly before death. This decrease is not associated with a destruction of erythrocytes by the virus or its metabolic products, such as is encountered in redwater (piroplasmosis) or anaplasmosis. In my opinion physical factors play the important rôle in producing these alterations. The increased water intake associated with hyperrhexia, tending to increased water ingestion, would tend towards the creation of hydraemia or dilution of the blood, leading to a decreased Hb. content per unit of blood, although the total Hb. would remain the same. On the other hand, the transudation of plasma into the peritoneal, pleural and pericardial cavities and the interstitial tissues of the lungs, especially if occurring rapidly at any particular stage of the disease, would tend towards a condition of a concentration of the blood. This transudation is probably a fairly rapid process; sheep killed in the earlier stages of the disease rarely show well-marked accumulations of transudate. Furthermore, the course of the disease is frequently so rapid that animals often survive only a short time after the onset of the temperature reaction. Where they survive one would expect an equally rapid return to the " mean " Hb. level by the absorption of the transudate, or of water into the blood, the osmotic forces tending to establish the *status quo*.

(*b*) In some cases there is no definite change in the Hb. in either direction (Cases II and VII). If the above conception is correct one could assume in explanation that either the analyses were not performed at the psychological moment as far as the onset of the transudation is concerned, or that the absorption of water into the blood occurred as rapidly as the loss of plasma through transudation.

(*c*) In Cases V and IX no distinct variations occurred beyond a rise in Hb. shortly before death.

Sugar (S).—The sugar content of the blood in heartwater shows a distinct increase in all cases studied, e.g., Case I, from 61–93 mgm. per cent, Case IX 56–156 mgm. per cent. In the majority of cases there is a decrease towards the end of the temperature reaction or just shortly before death in fatal cases.

This applies in both "laked" amd "unlaked" filtrates. Case VI provides an interesting exception—in the "laked" the increase is from ± 40 mgm. to 96 mgm. per cent., but in the "unlaked" only from ± 33–44 mgm. per cent., so that the "laked" contained over twice as much blood sugar at the peak point than the "unlaked." The decrease in several cases (III and VIII) continues to below the initial "mean" level. The increase is not in all cases absolute, i.e., does not necessarily proceed beyond the high side of normal, but the increase is evidenced by the gradual and definite rise from a lower level to a higher one. As previously stated the normal range lies between 45–55 mgm. per cent. although figures lower and above these limits are encountered, though relatively rarely. These figures agree closely with those of Völker (1929). In a case in which the "mean" lies at ± 35 mgm. per cent. an increase to 55 mgm. per cent. should in my opinion be looked upon as abnormal, provided of course that the 35 mgm. per cent. reflects the true "mean" for that animal under its present environment and on the given ration.

Total Nitrogen (*T.N.*).—The T.N. in about half the cases (I, III, IV, V, VI, VII, VIII) shows a decrease; in the remaining cases there are minor variations but not in any specific direction. A partial decrease in the T.N. is a priori to be expected owing to the transudation of plasma into the body cavities and lungs. This drain of nitrogenous material is, however, rapidly replaced, as can be noted in cases ending in recovery, where shortly after the return of temperature to normal the T.N. has reached its previous level. In Case I there is a decrease from 2·774 to 2·294 gm. N per cent., which is followed by a rise to 2·611 gm. N on the day prior to death.

Non-Protein Nitrogen (*N.P.N.*).—In the majority of cases an increase in the N.P.N. in both filtrates has been noted. The increases vary from about 15–36 mgm. N per cent. (Case II), 15–26·55 mgm. N per cent. (Case I), 20–30 mgm. N per cent. (Case III), 20–64 mgm. N per cent. (Case IV), 16–40 mgm. N per cent. (Case VII), 19–52 mgm. N per cent. (Case VIII) and 20–40 mgm. N per cent. (Case IX). Cases V and VI are interesting in that the "normal" level before injection of virus was on the high side and no distinct increases are noted. This N.P.N. retention follows the temperature reaction, returning to normal practically with cessation of the hyperthermic state. This is particularly well illustrated in Case VIII, where a severe and long drawn out temperature reaction is followed by a brief return to normal, whereafter a second less severe reaction sets in. The N.P.N. increases during the first reaction from 19–52 mgm. N per cent., drops to 20 mgm. N per cent. with drop of temperature to normal, and rises again to 25·50 mgm. N per cent. during the second reaction.

The U.N. is primarily responsible for this increase, the other constituent N fractions either remaining normal (A.A.N.) or showing a tendency to *decrease.* The total content and variations of the U.A.N. are too small appreciably to influence the N.P.N. in either direction. That, however, the U.N. is not solely responsible become evident by the observation that usually during the period of highest N.P.N. concentration, the R.N. (i.e., rest-nitrogen or *undetermined* nitrogen) represents in a number of cases from 5–9 mgm. N per cent. instead of the normal 2–4 mgm. N per cent. for "laked" and less for "unlaked" filtrates.

Urea Nitrogen (*U.N.*).—The U.N. has been found to be greatly increased in heartwater, e.g., Case II 4–22 mgm. N per cent., Case IV 7–32 mgm. N per cent., Case VII 4–24 mgm. N per cent., Case VIII 6·5–37 mgm. N per cent., Case IX 6–29 mgm. N per cent., the increase and decrease (with recovery cases) running parallel with the temperature reaction; the "mean" level being rapidly attained at the end of the hyperthermia. A high U.N. figure is generally associated with renal impairment or dysfunction. In heartwater there is in most cases found a fatty degeneration of the renal cortex, but I am inclined to consider the cardiac disturbance, as evinced by the cyanosis of mucous membranes, as the chief factor in the severe U.N. retention. According to this conception the cardiac condition leading to circulatory disturbances and thereby to renal dysfunction is of more significance in U.A. retention than the degeneration of the kidney. In two out of the three cases of recovery the U.N. remained at below 18 and 13 mgm. N per cent. respectively, in the third case the U.N. reached the high figure of 37 mgm. N per cent., and, as already mentioned, the recovery here was a matter of doubt for several days. It could theoretically be advanced that the virus plays some rôle in the production of the urea by toxicogenic protein decomposition, but in view of the rapid excretion of urea via the kidneys, this would have to be extensive before an accumulation in the blood could be expected. In such a case one would expect a more marked reduction in the protein nitrogen than is actually the case.

Uric Acid Nitrogen (*U.A.N.*).—No common factor can be traced through the data collected, there being a tendency towards an increase in some cases, the reverse in others, and in others again no change in either direction.

"*Total creatinine*" *Nitrogen* (*T.C.N.*).—The values found for T.C.N. are somewhat irregular and permit of no definite conclusions. On the whole there exists a tendency towards a decrease during heartwater, e.g., Case I 2·6–1·91 mgm. N per cent., Case IX 2·31–1·91 mgm. N per cent., Case X 2·23–1·19 mgm. N per cent., in "laked" and corresponding decreases in "unlaked" filtrates. In most cases where no decrease during the temperature reaction has occurred there is a slight increase again shortly before death. For this latter observation a possible explanation lies in the contracted agonal period, where the animal when down performs galloping movements with the limbs and exhibits severe tetanic spasms of the musculature resembling somewhat the syndrome encountered in cases of strychnine poisoning. It is generally accepted that creatinine is formed in the musculature and that its excretion is in some measure an expression of muscular activity. Separate determinations for creatine and creatinine were not done, and it is, therefore, not possible to state whether the creatine or the creatinine fraction, or both, are responsible for the decreases and increases found.

Amino-acid Nitrogen (*A.A.N.*).—No distinctive variations noticeable; in two cases a slight decrease prior to death is noted (Cases III and IX).

CONCLUSION.

In heartwater increases in the concentrations of sugar, N.P.N. and U.N. in both filtrates are recorded. The T.C.N. shows a tendency towards decreasing, with a rise in some cases just before death. The Hb. content varies, but is generally in the direction of a decrease.

B.—BLUETONGUE IN SHEEP (CATARRHAL FEVER).

The condition referred to here is the catarrhal fever or "Bluetongue" found in South Africa.

It has been defined as an inocuable disease of sheep principally affecting the mucous membranes of the mouth, nose, and intestines, very often accompanied with inflammation of the laminae of the feet. It is caused by an ultra-visible virus, probably transmitted from sheep to sheep by some flying insect. Animals which have recovered are immune and no longer carry the virus in the blood.

For more detailed information the articles listed in the bibliography at the end of this article may be consulted, the symptomatology being only very briefly described here.

Clinically abortive, acute and subacute forms are distinguished.

In the *abortive form* no symptoms, beyond a temperature reaction, can be seen.

Acute or subacute form.—With artificial infection the incubation period is generally 2-4 days, the hyperthermia lasting 5-7 days. Other symptoms are usually observed 7–10 days p.i.—generally salivation, swelling and catarrh of the buccal, mucous and nasal membranes and gums; occasionally also of the conjunctiva. Small sores may be noted on the lips. If more severe, an haemorrhagic inflammation may develop, the tongue becoming very swollen and brownish-red in colour, usually associated with a shiny greyish nasal discharge. In many cases a laminitis is observed. The animals lose in condition and may shed their wool (rare). The mortality varies– it is generally low but may be as high as 50 per cent.

(*a*) SELECTION AND TREATMENT OF THE SHEEP.

In the present researches nine sheep were utilised. All were artificially infected and all reacted. Blood was examined before infection and thereafter, particularly during the temperature reaction. The sheep used were all bastard Merinos, purchased in bluetongue-free areas and kept stabled. The ration given was the same as for the heartwater sheep (*vide*). In order to reduce wireworm infection to a minimum, the sheep received regular anthelminthic treatment with the Government Wireworm Remedy (monthly).

The strain of virus employed was a "field" strain, subinoculated into S. 34498 which succumbed to the infection, and although marked temperature reactions were obtained in the sheep, none died. Under the favourable experimental conditions, with its regular feeding, protection from the inclemencies of the weather, plentiful supply of water, etc., bluetongue is rarely fatal.

(*b*) CHEMICAL METHODS OF ANALYSES AND TECHNIQUE.

These are the same as those referred to under "Heartwater." In the arrangement and discussion the same general plan as for the previous disease has been adhered to.

(*c*) EXPERIMENTAL DATA.

(1) *Normal Range of Constituents.*

For the compilation of the following only data obtained from the sheep used for the bluetongue experiments *prior to* infection have been utilised. These data are additional to those tabulated under "Heartwater" and, although they agree with them closely, they are in general on a somewhat lower level. The former data were gathered during September to November, 1931, the latter during June to August, 1932, and the existing differences represent seasonal variations, there being a tendency for a progressive decrease in the concentrations of the various constituents during the winter. The present summary only represents twelve analyses of sheep blood of various ages, and for data comprising a much larger number of analyses of the normal blood of sheep under the same conditions I would refer to the work done by Hamersma (1933).

Haemoglobin.

Minimum–maximum variation: 9·1–16·5 gm. per cent.

Average: 13·0 gm. Hb. per cent.

Sugar.

Minimum–maximum variation "laked": 37–55·5 mgm. per cent.

Average "unlaked": 47·36 mgm. per cent.

Minimum–maximum variations "unlaked": 31·3–50·5 mgm. per cent.

Average "unlaked": 39·1 mgm. per cent.

Percentage differences between "laked" and "unlaked" 10·3–27·6 per cent.

Average percentage difference: 17·4 per cent.

Total Nitrogen.

Minimum–maximum variation: 2·4–3·3 gm. N per cent.

Average: 2·8 gm. N per cent.

Non-Protein Nitrogen.

Minimum–maximum variations "laked": 13·6–20 mgm. N per cent.

Average "laked": 16·5 mgm. N per cent.

Minimum–maximum variations "unlaked": 9·4–15·3 mgm. N per cent.

Average "unlaked": 12·5 mgm. N per cent.

Percentage differences between "laked" and "unlaked" 9·7–33·2 per cent.

Average percentage difference: 25 per cent.

Urea Nitrogen.

Minimum–maximum variation "laked": 3·1–7·3 mgm. N per cent.

Average "laked": 4·9 mgm. N per cent.

Minimum–maximum variation "unlaked" 3·0–7·3 mgm. N per cent.

Average "unlaked": 4·7 mgm. N per cent.

Percentage difference between "laked" and "unlaked" average 4·1 per cent.

Total Creatinine Nitrogen.

Minimum–maximum variations "laked": 1·67–2·35 mgm. N per cent.

Average "laked": 2·08 mgm. N per cent.

Minimum–maximum variations "unlaked": 1·43–2·00 mgm. N per cent.

Average "unlaked": 1·72 mgm. N per cent.

Percentage difference between "laked" and "unlaked" average 17·3 per cent.

Uric Acid Nitrogen.

Minimum–maximum variations "laked": 0·15–0·26 mgm. N per cent.

Average "laked": 0·22 mgm. N per cent.

Minimum–maximum variations "unlaked": 0·10–0·22 mgm. N per cent.

Average "unlaked": 0·13 mgm. N per cent.

Percentage difference between "laked" and "unlaked" average 41 per cent.

Amino-acid Nitrogen.

Minimum–maximum variations "laked": 4·1–6·1 mgm. N per cent.

Average "laked": 5·30 mgm. N per cent.

Minimum–maximum variations "unlaked": 2·72–3·90 mgm. N per cent.

Average "unlaked": 3·20 mgm. N per cent.

Percentage difference between "laked" and "unlaked" average 40 per cent.

Rest Nitrogen.

Minimum–maximum variations "laked": 2·65–6·73 mgm. N per cent.

Average "laked": 4·13 mgm. N per cent.

Minimum–maximum variations "unlaked": 1·54–3·58 mgm. N per cent.

Average "unlaked": 2·33 mgm. N per cent.

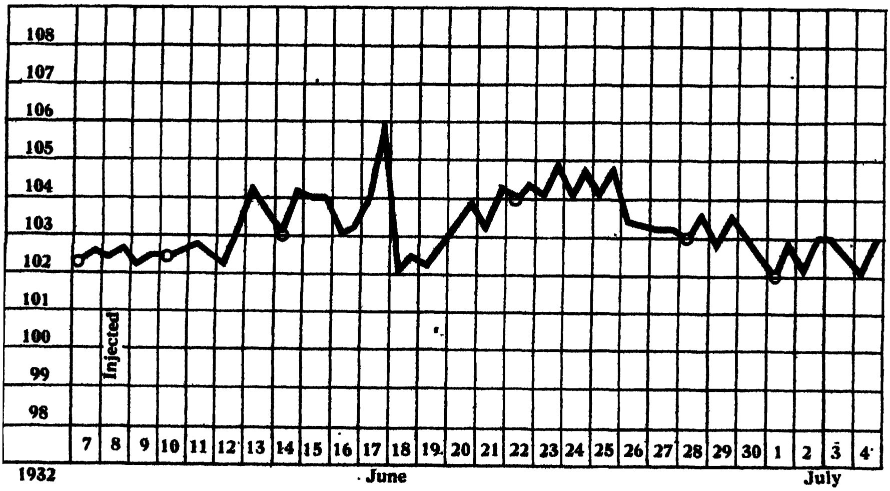
Percentage difference between "laked" and "unlaked" average 43·6 per cent.

(2) *BLUETONGUE DATA.*

CASE I.

Sheep 25142.

Temperature Chart I.



History.—A six-tooth ewe, carrying about 2 ins. wool, in good condition. Placed on temperature 17/5/32, and injected subcutaneously with 2 c.c. blood on 8/6/32. The reaction was severe but not in as much as the hyperthermia was concerned but rather in its effect on the animal, which showed cyanosis of the buccal and nasal mucous membranes and laminitis. Furthermore, as a result of this infection it shed its wool completely and lost condition. Discharged 27/8/32.

TABLE 1.—S. 25142. *Bluetongue.*

Date		7/6/32.	10/6/32.	14/6/32.	22/6/32.	28/6/32.	1/7/32.	5/7/32.	14/7/32.	20/7/32.
Time		—	—	—	—	—	—	—	—	—
Temperature Reaction		N	N	P.I.N.	P.I.N.	R	R	N	N	N
***Haemoglobin* gm. %**		15·98	14·95	16·54	11·39	8·49	8·49	9·32	9·81	9·94
***Sugar* mgm. %**	L	44·64	—	—	117·64	63·69	59·52	34·60	65·79	80·65
	U	34·48	—	—	111·10	44·05	53·76	29·94	59·88	68·03
***N.P.N.* mgm. %**	L	20·63	16·30	22·39	66·60	26·31	18·75	21·43	11·32	13·82
	U	13·63	10·82	15·82	53·55	23·44	14·71	18·36	8·33	12·00
***Total N.* gm. %**		3·074	3·00	3·032	2·416	2·220	2·322	2·360	2·318	2·290
***Coag. N* gm. N %**	L	3·053	2·984	3·009	2·350	2·194	2·313	2·339	2·307	2·276
	U	3·060	2·989	3·016	2·362	2·197	2·317	2·342	2·310	2·278
Urea mgm. N %	L	6·69	3·83	9·87	49·00	15·53	7·33	11·27	2·51	6·41
,, U %		14·07	7·98	20·79	102·90	32·55	15·33	23·73	5·25	13·44
mgm. N %	U	6·97	3·69	9·42	43·44	15·39	7·26	10·56	2·33	6·12
,, U %		14·49	7·77	19·74	91·14	32·34	15·33	22·26	4·83	12·81
Total Creatinine mgm. N %	L	2·27	—	—	4·02	2·57	2·27	1·87	1·87	1·78
,, TC %		6·16	—	—	10·80	6·96	6·16	5·02	5·02	4·80
mgm. N %	U	1·82	—	—	2·76	2·01	1·49	1·44	1·54	1·54
,, TC %		4·90	—	—	7·44	5·40	4·00	3·86	4·16	4·16
Uric acid mgm. N %	L	0·25	—	—	0·22	0·25	—	0·23	0·21	0·19
,, UA %		0·76	—	—	0·67	0·76	—	0·69	0·64	0·58
mgm. N %	U	0·12	—	—	0·11	0·11	—	0·18	0·18	0·14
		0·35	—	—	0·32	0·33	—	0·55	0·53	0·41
***Amino acid* mgm. N %**	L	5·69	5·56	5·56	5·60	5·18	5·83	4·32	4·24	4·24
	U	2·92	2·92	3·29	3·67	2·92	3·41	2·54	2·72	2·50
***Rest N.* mgm. N %**	L	6·73	6·91*	6·96*	6·76	2·78	3·32†	3·74	2·49	1·20
	U	1·80	4·21*	3·11*	3·57	3·00	2·55†	3·74	1·56	1·70
Plasma		n.u.	n.u.	n.u.	n.u.	n.u.	n.u.	n.u.	n.u.	n.u.

* Includes "Total Creatinine Nitrogen" and "Uric Acid Nitrogen."   † Includes "Uric Acid. N."

Main Features of Analytical Data.

Hb.—A drop from 15·73–8·49 gm. % is noted, the decrease coinciding with the onset of the fever reaction. The return to its previous Hb. level was rather gradual, 9·94 gm. % only being reached after about four weeks.

Sugar.—Shows a striking rise from 45–117·64 mgm. % and from 34·5–111·1 mgm. % for "laked" and "unlaked" filtrates, respectively

N.P.N.* and *U.N.—These are grouped together since the increase in N.P.N. is due to the increased U.N. which goes up from approximately 5–49 mgm. N %, i.e. nearly 1000 %. The N.P.N. goes up to 66 and 54 mgm. N % for "laked" and "unlaked" filtrates, respectively.

T.C.N.—During the reaction a slightly higher level for T.C.N. is recorded.

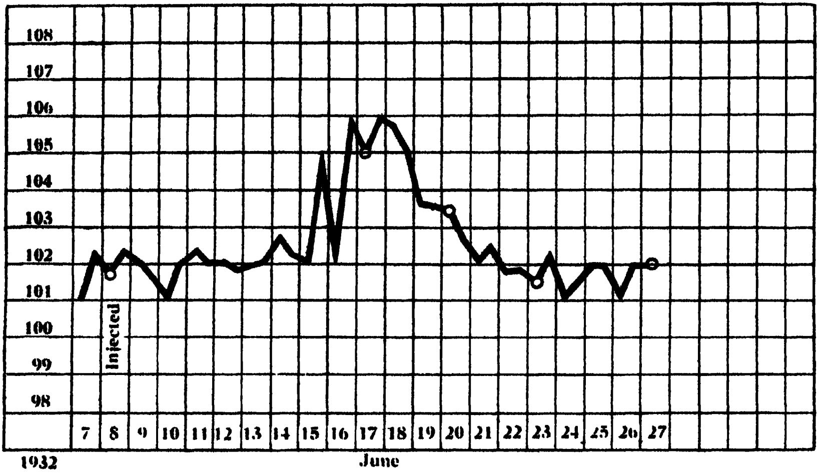
T.N.—Decreases proportionally to the drop in the Hb. value.

U.A.N.*, *A.A.N.* and *R.N.—Nothing unusual.

CASE II.

Sheep 22204.

Temperature Chart II.

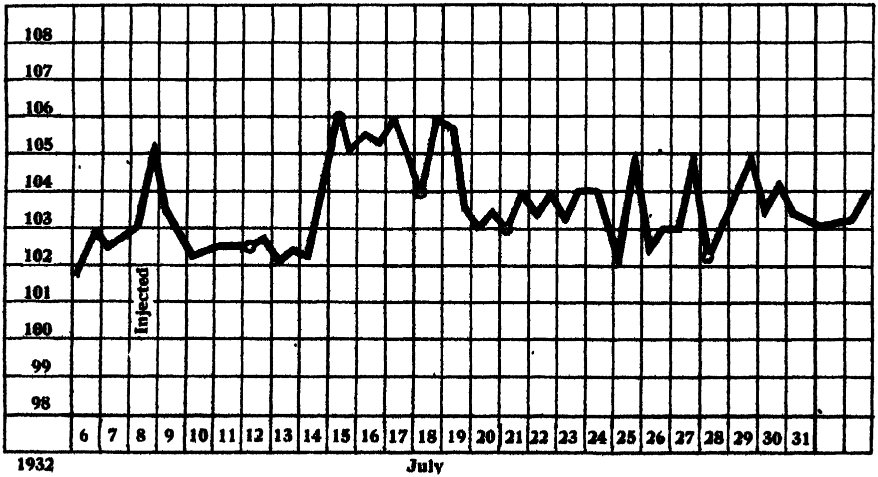


History.—A four-tooth ewe, carrying 2 ins. wool, in good condition. Placed on temperature 17/5/32 and injected subcutaneously with 2 c.c. virulent blood on 8/6/32. After an incubation period of six days the temperature rose. Towards the end of the reaction the animal appeared rather dull and listless and showed slight cyanosis of the buccal mucous membrane. It made an uneventful recovery. Discharged 15/7/32.

CASE III.

S. 34896.

Temperature Chart III.



History.—A two tooth hamel, 3 ins. wool, in good condition. Placed on temperature 18/5/32 and injected subcutaneously with 2 c.c. blood on 8/7/32. Incubation period five days. The animal showed an injection of the buccal mucous membranes and slight laminitis, but recovered very quickly from these symptoms. Discharged 27/8/32.

TABLE 2.—S. 22204. *Bluetongue.*

Date *Time*		19/5/32. —	8/6/32. —	17/6/32. —	20/6/32. 10.30 a.m.	23/6/32. —	27/6/32. —	30/6/32. —	5/7/32. —	14/7/32. —	23/7/32. 7.15 a.m.
Temperature Reaction		N	N	R	R	N	N	N	N	N	N
Haemoglobin gm. %		16·50	14·49	15·53	18·20	14·28	13·31	12·92	13·48	12·79	12·79
Sugar mgm. %	L U	42·92 38·46	44·35 39·84	50·00 44·30	— —	53·48 50·00	46·08 42·01	45·05 40·32	49·26 40·82	43·10 38·17	49·75 44·05
Total N. gm. %		3·256	2·906	3·092	3·144	2·843	2·640	2·689	2·696	2·590	2·759
N.P.N. mgm. %	L U	15·46 12·10	13·63 9·38	18·40 16·10	20·00 18·75	25·86 21·43	15·00 12·50	14·71 11·36	15·41 11·25	13·04 9·68	15·00 10·71
Coag. N gm. N %	L U	3·241 3·244	2·892 2·897	3·074 3·076	3·124 3·125	2·816 2·822	2·625 2·628	2·674 2·678	2·681 2·685	2·557 2·560	2·744 2·748
Urea mgm. N %.... ,, U %.... mgm. N %.... ,, U %....	L U 	4·13 8·61 4·00 8·40	3·13 6·51 3·00 6·30	8·25 17·43 8·00 16·80	12·60 26·46 11·99 25·20	13·29 27·93 12·69 26·67	5·76 12·18 5·49 11·55	4·71 9·87 4·65 9·87	4·26 9·03 4·14 8·61	3·88 8·19 3·55 7·56	3·85 8·19 4·00 8·40
Total Creatinine mgm. N %.... ,, TC %... mgm. N %.... ,, TC %...	 L U 	1·82 4·90 1·49 4·00	2·17 5·84 2·01 5·40	2·23 6·00 1·91 5·14	— — — —	— — 1·61 4·32	2·11 5·68 1·86 5·62	2·23 6·00 1·64 4·40	2·10 5·68 1·44 3·86	2·27 6·16 2·17 5·84	2·10 5·68 1·92 4·90
Uric acid mgm. N %.... ,, UA %.. mgm. N %.... ,, UA %..	L U 	0·23 0·69 0·18 0·53	0·15 0·45 0·09 0·27	0·14 0·41 0·10 0·29	— — — —	0·13 0·40 0·07 0·20	0·15 0·44 0·09 0·28	0·27 0·80 — —	0·20 0·61 0·19 0·57	0·17 0·50 0·13 0·40	0·11 0·33 0·07 0·20
Amino acid mgm. N %...	L U	6·09 3·89	4·11 2·74	4·64 3·11	4·46 2·70	5·30 3·50	4·83 3·04	4·38 2·72	4·64 3·04	4·36 3·11	3·89 2·80
Rest N. mgm. N %......	L U	3·19 2·56	4·07 1·54	3·14 3·00	2·94* 4·16*	7·14† 3·57	2·15 2·02	3·12 2·35‡	4·12 2·44	2·36 4·28	5·05 2·04
Plasma		n.u.	n.u.	n.u.	n.u.	n.u.	n.u.	n.u.	n.u.	n.u.	n.u.

* Includes " Total Creatinine " and " Uric Acid Nitrogen." † Includes " Total Creatinine." ‡ Includes " Uric Acid."

Main Features of Analytical Data.

Hb.—Gradually decreased from 15·53–12·79 gm. %, the decrease coinciding with the return of the temperature to normal.

N.P.N.—Shows an increase from approximately 15–26 % and from 12–21 mgm. N % for " laked " and " unlaked " filtrates, respectively. The return to normal occurred a few days after the cessation of the febrile reaction.

U.N.—An increase from 3·5–13 mgm. N % is noted.

U.A.N.—A distinct tendency towards a decreased level during the febrile stage in both filtrates occurred.

T.N.—Decreases from ± 3–2·6 gm. %, this drop coinciding with the alteration in the Hb. level.

Sugar, T.C.N., A.A.N., R.N.—Nothing unusual.

TABLE 3.—S. 34896. *Bluetongue.*

			30/6/32.	12/7/32.	15/7/32.	18/7/32.	21/7/32.	28/7/32.	5/8/32.
Date *Time*			—	—	—	—	—	—	—
Temperature Reactions			N	P.I.N.	R	R	R	R	N
Haemoglobin gm. %			14·08	14·28	14·08	13·50	16·50	12·94	14·28
Sugar mgm. %		L	46·08	43·29	71·43	51·02	54·35	44·05	46·51
		U	35·21	32·16	62·11	45·05	48·08	35·97	39·68
T.N. gm. %			2·864	2·710	2·724	2·605	2·655	2·696	2·717
N.P.N. mgm. %		L	18·90	23·69	21·43	18·52	18·63	17·02	16·31
		U	15·28	17·44	14·28	12·66	13·39	12·00	11·53
C.N. gm. N %		L	2·845	2·686	2·703	2·586	2·636	2·679	2·701
		U	2·849	2·693	2·710	2·592	2·642	2·684	2·705
Urea	mgm. N %	L	7·33	10·11	8·39	7·16	6·40	5·25	4·40
	,, U %		15·33	21·21	17·64	15·12	13·44	11·13	9·24
	mgm. N %	U	7·33	9·87	8·09	7·06	6·27	5·19	4·13
	,, U %		15·33	20·79	17·01	14·91	13·23	10·92	8·61
T.C.	mgm. N %	L	2·23	2·35	2·42	2·17	2·10	2·23	2·17
	,, TC %		6·00	6·36	6·54	5·84	5·68	6·00	5·84
	mgm. N %	U	1·51	1·78	1·82	1·78	1·91	1·75	1·82
	,, TC %		4·08	4·80	4·90	4·80	5·14	4·70	4·90
Uric acid	mgm. N %	L	—	0·27	0·25	0·22	0·23	0·17	0·23
	,, UA %		—	0·80	0·74	0·66	0·68	0·52	0·69
	mgm. N %	U	—	0·11	0·12	0·11	0·13	0·07	0·11
	,, UA %		—	0·32	0·35	0·33	0·40	0·22	0·32
Amino acid mgm. N %		L	5·58	6·36	5·76	4·67	4·67	6·09	6·09
		U	3·33	3·68	3·18	2·54	3·04	4·00	3·85
R.N. mgm. N %		L	3·86*	4·60	4·61	4·30	5·23	3·28	3·42
		U	2·46*	2·06	1·07	1·17	2·04	1·00	1·62
Plasma			n.u.	n.u.	n.u.	n.u.	n.u.	n.u.	n.u.

* Includes "Uric Acid N."

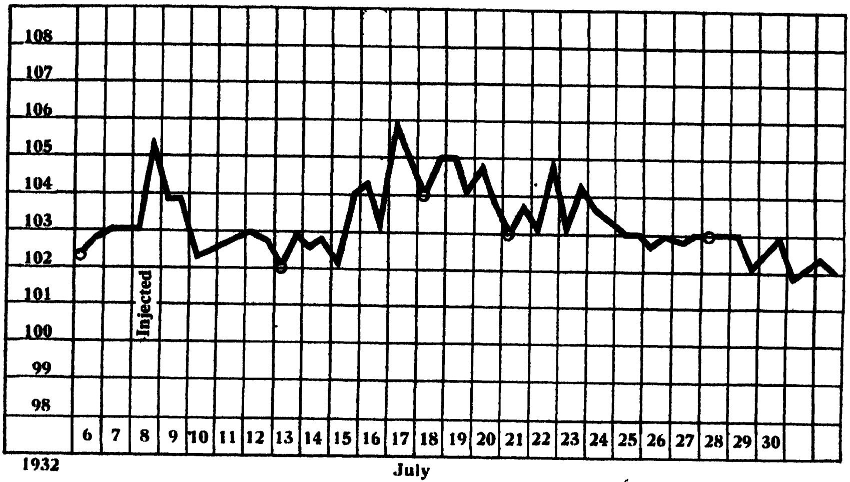
Main Features of Analytical Data.

Sugar., N.P.N., and *U.N.*—Slight tendency towards a higher level which, however, is not above the upper limits of normal.
A.A.N.—A drop during the hyperthermia is noted.
Hb., *T.N.*, *U.A.N.*, *T.C.N.*, and *R.N.*—Nothing unusual.

CASE IV.

S. 34898.

Temperature Chart IV.

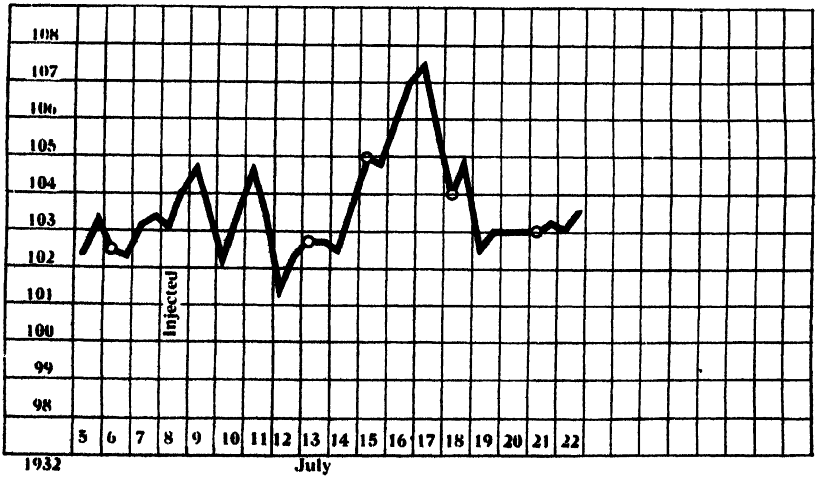


History.—A four tooth hamel, 3½ ins. wool, in good condition. Placed on temperature 19/5/32 and injected subcutaneously with 2 c.c. blood on 8/7/32. After an incubation period of seven days an irregular mild fever reaction supervened. From the 23rd–26th a slight laminitis was noted. It made an uneventful recovery. Discharged 27/8/32.

CASE V.

S. 34885.

Temperature Chart V.



History.—A four-tooth hamel, 3½ ins. wool, in good condition. Drafted into experiment 18/5/32 and injected subcutaneously with 2 c.c. blood on 8/7/32. Incubation period five days, and beyond a hyperthermia no other symptoms were shown. Discharged on 27/8/32.

TABLE 4.

S. 34898. *Bluetongue.*

Date............. *Time*.............	6/7/32.	13/7/32.	18/7/32.	21/7/32.	28/7/32.	5/8/32.
Temp. R.........	N	P.I.N.	R	R	N	N
Hb. gm. %.......	12·59	11·24	11·67	12·28	11·14	11·14
Sugar mgm. % L	41·49	48·08	58·14	48·31	42·92	47·85
U	32·26	37·45	47·17	38·02	35·21	39·68
Total N. gm. %..	2·598	2·430	2·332	2·360	2·444	2·479
N.P.N. mgm. % L	17·65	15·79	16·30	13·63	13·65	19·23
U	14·54	10·71	11·07	10·79	10·71	15·00
Coag. N. L	2·580	2·414	2·316	2·346	2·430	2·460
gm. N % U	2·583	2·419	2·321	2·349	2·433	2·464
Urea mgm. N % L	7·20	4·10	5·45	4·48	3·88	7·51
„ U %	15·12	8·61	11·55	9·45	8·19	15·75
mgm. N % U	7·03	3·94	5·45	4·56	3·40	7·33
„ U %	14·70	8·19	11·55	9·66	7·14	15·33
T.C. mgm. N % L	1·98	2·01	1·49	2·01	2·50	2·35
„ TC %	5·32	5·40	4·00	5·40	6·74	6·36
mgm. N % U	1·67	1·71	1·82	1·98	2·35	2·04
„ TC %	4·50	4·60	4·90	4·32	6·36	5·50
Uric acid						
mgm. N % L	0·25	0·19	0·21	0·19	0·13	0·20
„ UA %	0·76	0·56	0·64	0·57	0·40	0·60
mgm. N % U	0·12	0·08	0·12	0·14	0·08	0·11
„ UA %	0·36	0·25	0·36	0·41	0·24	0·33
Amino acid L	5·56	5·60	4·00	4·12	5·18	5·64
mgm. N % U	3·77	3·67	2·52	2·92	3·72	3·89
Rest N. L	2·65	3·89	5·15	2·83	2·96	3·53
mgm. N % U	1·95	1·31	1·18	1·79	1·16	1·63
Plasma...........	n.u.	n.u.	n.u.	n.u.	n.u.	n.u.

Main Features of Analytical Data.

Except for a tendency towards a slight decrease in the A.A.N. level during the febrile reaction, no other blood changes occurred.

TABLE 5.

S. 34885. *Bluetongue.*

Date............. *Time*.............	6/7/32. —	13/7/32. —	15/7/32. —	18/7/32. —	21/7/32. —	28/7/32. —
Temp. R..........	N	P.I.N.	R	R	N	N
Hb. gm. %.......	10·35	9·81	9·94	11·14	8·88	9·40
Sugar mgm. %. L	48·31	44·84	62·50	60·24	51·28	52·63
U	40·16	33·78	49·02	49·75	46·95	45·25
Total N. gm. %..	2·542	2·402	2·290	2·304	2·059	2·220
N.P.N. mgm. % L	17·04	17·65	17·85	23·20	20·00	15·00
U	12·94	13·57	13·04	16·76	16·48	11·11
Coag. N. L	2·525	2·384	2·272	2·281	2·039	2·205
gm. N % U	2·529	2·388	2·277	2·287	2·039	2·205
Urea mgm. N % L	4·61	5·37	6·46	11·50	10·43	4·33
„ U %	9·66	11·34	13·65	24·15	21·84	9·03
mgm. N % U	4·26	5·06	6·69	11·43	10·03	4·19
„ U %	9·03	10·71	14·07	23·94	21·00	8·82
Total Creatin.						
mgm. N % L	2·17	2·01	2·17	2·57	1·91	2·50
„ TC %	5·84	5·70	5·84	6·96	5·14	6·74
mgm. N % U	1·91	1·78	1·91	1·82	1·86	2·10
„ TC %	5·14	4·80	5·14	4·90	5·02	5·64
Uric acid						
mgm. N % L	0·26	0·20	0·22	0·23	0·21	0·15
„ UA %	0·70	0·60	0·65	0·69	0·64	0·46
mgm. N % U	0·12	0·10	0·12	0·11	0·12	0·08
„ UA %	0·36	0·30	0·36	0·34	0·36	0·25
Amino acid L	5·60	6·03	5·38	3·84	3·89	5·60
mgm. N % U	4·11	3·91	3·59	2·32	2·69	3·65
Rest N. L	4·40	4·04	3·62	5·06	3·56	2·42
mgm. N % U	2·54	2·72	0·75	1·08	1·70	1·09
Plasma...........	n.u.	n.u.	n.u.	n.u.	n.u.	n.u.

Main Features of Analytical Data.

Sugar.—Shows a slight rise.

N.P.N.—Shows a slight increase from approximately 17–23 mgm. N % and 13–17 mgm. N %.

U.N.—An increase from 4·5–11 mgm. % is observed.

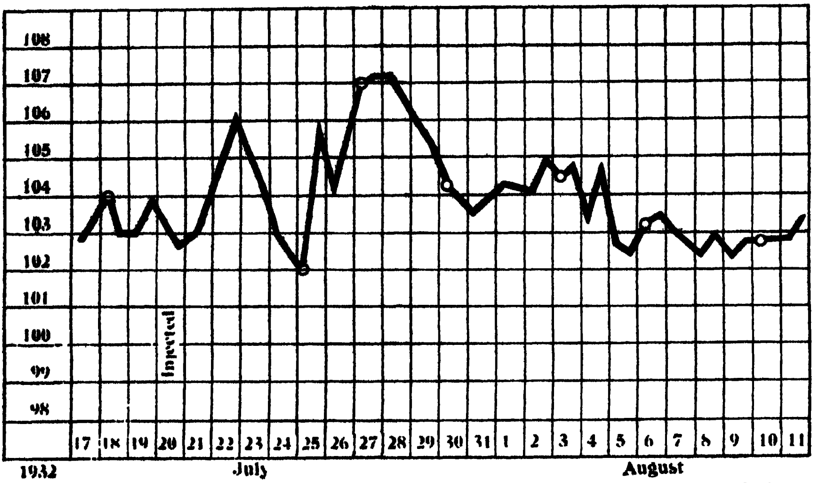
A.A.N.—Slight decrease during the temperature reaction.

Hb., T.C.N., U.A.N., R.N., T.N.—Nothing unusual.

CASE VI.

S. 34872.

Temperature Chart VI.

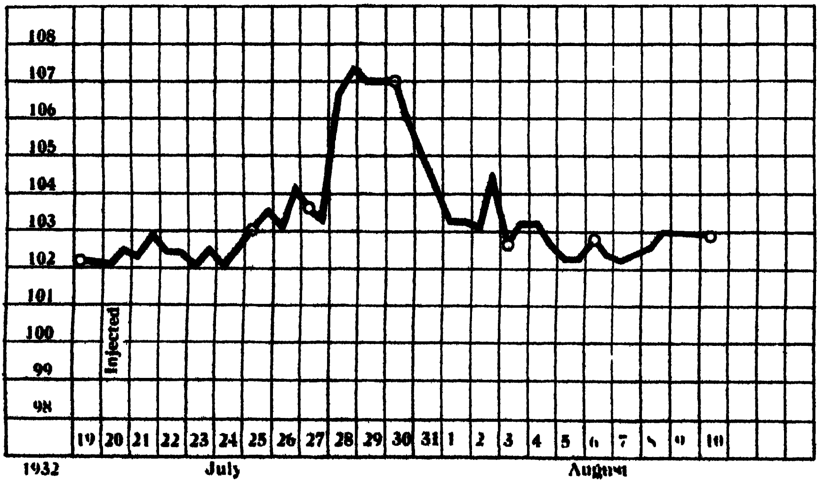


History.– A two-tooth hamel, 3½ ins. wool, in good condition. Injected subcutaneously 2 c.c. blood on 27/7/32, and after an incubation period of two days a severe and prolonged hyperthermia supervened. Towards the end of the reaction the animal became dull and went down, but showed no other symptoms. Discharged 27/8/32.

CASE VII.

S. 34870.

Temperature Chart VII.



History.—A two-tooth hamel, 3 ins. wool, good condition. Placed on emperature 18/5/32 and injected subcutaneously with 2 c.c. blood on 20/7/32. ncubation period four days. Except for a mild laminitis and for a slight eddening of the buccal mucous membrane, recovery was uneventful.

Table 6.—S. 34872. *Bluetongue.*

			8/7/32.	18/7/32.	25/7/32.	27/7/32.	30/7/32.	3/8/32.	6/8/32.	10/8/32.
Time			—	—	—	—	—	—	7.15 a.m.	—
Temperature Reaction			N	N	R	R	R	R	N	N
Haemoglobin gm. %			14·08	13·87	11·53	11·92	11·94	10·27	11·53	10·87
Sugar mgm. %		L	41·49	37·04	49·26	48·31	75·19	71·43	46·51	47·40
		U	33·90	31·25	44·05	41·15	59·88	62·11	41·66	—
Total N. gm. %			2·752	2·780	2·444	2·472	2·434	2·318	2·325	2·400
N.P.N. mgm. %		L	15·00	15·00	13·04	15·87	17·97	20·00	17·65	15·40
		U	12·00	10·71	10·00	12·05	14·91	15·79	13·04	—
Coag. N gm. N %		L	2·737	2·765	2·431	2·456	2·416	2·298	2·307	2·385
			2·740	2·769	2·434	2·460	2·419	2·302	2·312	—
Urea	mgm. N %	L	5·45	4·19	3·00	6·40	9·52	9·00	7·00	5·25
	„ U %		11·55	8·82	6·30	13·44	19·95	18·90	14·70	—
	mgm. N %	U	5·15	4·13	3·55	5·97	9·25	9·00	6·84	—
	„ U %		10·92	8·61	7·56	12·60	19·53	18·90	14·20	—
Total Creatinine	mgm. N %	L	1·67	1·91	2·23	2·04	2·10	2·27	2·01	2·27
	„ TC %		4·50	5·14	6·00	5·50	5·68	6·16	5·40	6·16
	mgm. N %	U	1·43	1·61	1·91	1·67	1·78	1·98	1·61	—
	„ TC %		3·86	4·32	5·14	4·50	4·80	5·32	4·32	—
Uric acid	mgm. N %	L	0·20	0·19	0·22	0·19	0·15	0·23	0·20	0·15
	„ UA %		0·60	0·56	0·67	0·57	0·44	0·68	0·60	0·46
	mgm. N %	U	0·12	0·11	0·16	0·11	0·10	0·15	0·14	—
	„ UA %		0·36	0·34	0·47	0·33	0·29	0·46	0·41	—
Amino acid mgm. N %		L	4·24	4·97	4·38	3·84	4·52	5·00	5·56	5·18
		U	2·92	2·72	2·98	2·64	2·72	3·33	3·68	—
Rest N. mgm. N %		L	3·44	3·74	2·21	2·40	1·68	3·50	2·88	2·55
		U	2·38	2·14	1·40	1·66	1·06	1·73	1·43	—
Plasma			n.u.	n.u.	n.u.	n.u.	n.u.	n.u.	n.u.	n.u.

Main Features of Analytical Data.

Sugar.—Increases to 75 mgm. % during the latter part of the febrile reaction.
N.P.N., *U.N.*—Show relatively slight, but distinct, increase during reaction.
T.C.N., *U.A.N.*, *A.A.N.*, *T.N.*, *R.N.*—Nothing unusual.

TABLE 7.—S. 34870. *Bluetongue.*

Date			19/7/32.	25/7/32.	27/7/32.	30/7/32.	3/8/32.	6/8/32.	10/8/32.
Time			—	—	—	—	—	—	—
Temperature Reaction			N	P.I.N.	P.I.N.	R	R	N	N
Haemoglobin gm. %			11·39	13·31	13·12	12·79	13·31	11·39	11·14
Sugar mgm. %		L	51·28	55·55	59·17	62·50	55·55	57·80	40·00
		U	42·92	41·15	47·62	53·76	44·84	46·51	—
Total N gm. %			2·437	2·738	2·696	2·514	2·402	2·435	2·394
N.P.N. mgm. %		L	18·18	16·66	14·85	23·08	18·75	17·14	18·75
		U	12·14	10·98	11·03	15·98	13·63	18·35	—
Coag. N. gm. N %		L	2·419	2·721	2·681	2·491	2·383	2·418	2·375
		U	2·425	2·727	2·685	2·498	2·388	2·425	—
Urea	mgm. N %	L	5·25	4·88	4·48	10·43	6·55	4·26	6·00
	,, U %		10·93	10·29	9·45	21·84	13·86	9·03	12·60
	mgm. N %	U	5·35	3·69	4·40	10·17	6·27	4·00	—
	,, U %		11·34	7·77	9·24	21·42	13·23	8·40	—
T.C.	mgm. N %	L	2·23	2·50	2·23	2·27	1·82	1·75	1·87
	,, TC %		6·00	6·74	6·00	6·16	4·90	4·70	5·14
	mgm. N %	U	2·01	1·75	1·71	2·04	1·49	1·49	—
	,, TC %		5·40	4·70	4·60	5·50	4·00	4·00	—
Uric acid	mgm. N %	L	0·22	0·26	0·21	0·20	0·27	0·27	0·17
	,, UA %		0·67	0·78	0·64	0·61	0·80	0·82	0·50
	mgm. N %	U	0·12	0·19	0·11	0·08	0·16	0·13	—
	,, UA %		0·36	0·50	0·33	0·24	0·47	0·40	—
Amino acid mgm. N %		L	5·38	5·18	6·38	5·38	5·60	5·83	5·51
		U	3·04	3·33	3·50	2·92	4·06	3·59	—
R.N. mgm. N %		L	5·10	3·84	2·75	4·80	4·51	5·03	5·20
		U	1·62	2·04	1·31	0·77	1·45	1·14	—
Plasma			n.u.	n.u.	n.u.	n.u.	n.u.	n.u.	n.u.

Main Features of Analytical Data.

Sugar.—Slight tendency to rise.

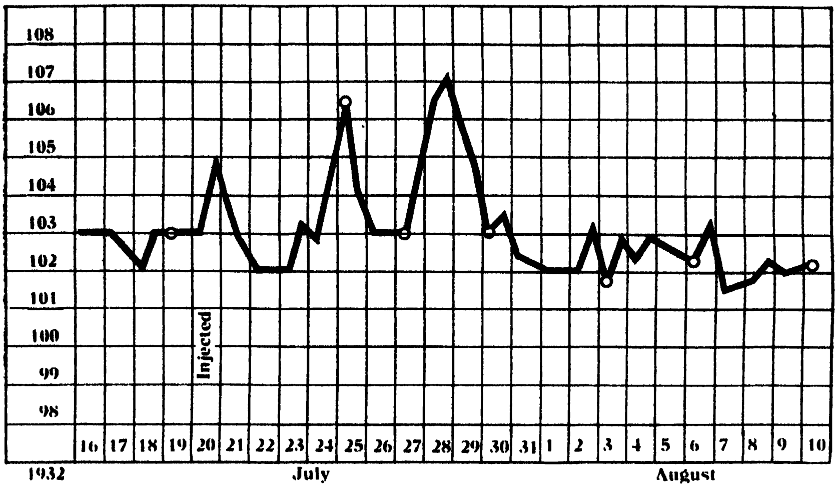
N.P.N. and *U.N.*—Both show a distinct increase, e.g., N.P.N. from 16–23 and from 11–16 mgm. N %, and U.N. from 4–10·4 mgm. N %, with a rapid return to normal after the temperature reaction has ended.

Hb., T.C.N., U.N.N., A.A.N., R.N., and *T.N.*—Nothing unusual.

Case VIII.

S. 34900.

Temperature Chart VIII.

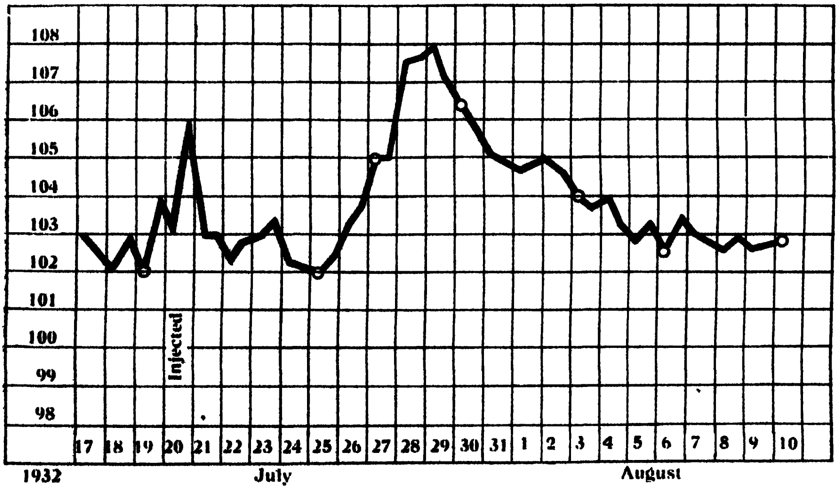


History.—A four-tooth hamel, 3 ins. wool, good condition. Was placed on temperature 18/5/32 and injected subcutaneously on 20/7/32 with 2 c.c. blood. After an incubation period of four days a temperature reaction set in, but apart from this and a slight dullness, the animal showed no other symptoms. Discharged 27/8/32.

Case IX.

S. 34912.

Temperature Chart IX.



History.—A two-tooth hamel, 3½ ins. wool, in good condition. Drafted into experiment 19/5/32 and injected subcutaneously with 2 c.c. blood on 20/7/32. After an incubation period of five days a severe hyperthermia set in, reaching 108° in 72 hours, but, except for a slight dullness and loss of appetite, no other symptoms were noted. Discharged 27/8/32.

TABLE 8.—S. 34900. *Bluetongue.*

			19/7/32.	25/7/32.	27/7/32.	30/7/32.	3/8/32.	6/8/32.	10/8/32.
Time			—	—	—	—	—	7.15 a.m.	—
Temperature Reactions			N	R	R	R	N	N	N
Haemoglobin gm. %			11·24	10·76	11·14	12·13	10·99	11·96	11·24
Sugar mgm. %		L	56·18	64·94	60·61	66·67	59·52	58·14	45·60
		U	50·00	55·50	49·50	54·05	51·55	49·50	—
T.N. gm. %			2·451	2·374	2·493	2·535	2·409	2·640	2·520
N.P.N. mgm. %		L	16·48	17·35	16·66	22·90	19·73	17·65	15·80
		U	11·28	14·00	10·86	16·04	15·79	11·53	—
Coag. N. gm. N %		L	2·435	2·357	2·476	2·512	2·389	2·622	2·504
		U	2·440	2·360	2·482	2·519	2·393	2·628	—
Urea	mgm. N %	L	3·27	5·45	4·40	9·42	7·00	4·71	4·13
	„ U %		6·93	7·35	9·24	19·74	14·70	9·87	8·67
	mgm. N %	U	3·00	5·35	4·13	9·00	6·67	4·88	—
	„ U %		6·30	11·34	8·67	18·90	14·07	10·29	—
Total Creatinine	mgm. N %	L	2·35	2·42	2·23	2·23	2·10	2·10	2·17
	„ TC %		6·36	6·54	6·00	6·00	5·68	5·68	5·84
	mgm. N %	U	1·82	2·35	1·67	1·67	1·82	1·71	—
	„ TC %		4·90	6·36	4·50	4·50	4·90	4·60	—
Uric acid	mgm. N %	L	0·22	0·26	0·22	0·20	0·25	0·29	0·20
	„ UA %		0·67	0·78	0·67	0·59	0·74	0·86	0·60
	mgm. N %	U	0·15	0·20	0·13	0·11	0·15	0·14	—
	„ UA %		0·45	0·59	0·39	0·32	0·45	0·41	—
Amino acid mgm. N %		L	5·79	4·83	5·26	5·98	5·96	6·22	5·15
		U	3·50	3·33	3·41	3·81	4·24	4·00	—
R.N. mgm. N %		L	4·85	4·39	4·55	5·07	4·42	4·33	4·15
		U	2·81	2·77	1·52	1·45	2·91	0·80	—
Plasma			n.u.	n.u.	n.u.	n.u.	n.u.	n.u.	n.u.

Main Features of Analytical Data.

Sugar, N.P.N. and *U.N.*—These constituents have a tendency to rise slightly during the febrile reaction, reaching concentration just over the upper level of the normal range.

Hb., T.C.N., U.A.N., A.A.N., R.N. and *T.N.*—Nothing usual.

TABLE 9. S. 34912. *Bluetongue.*

			19/7/32.	25/7/32.	27/7/32.	30/7/32.	3/8/32.	6/8/32.	10/8/32.
Time			—	—	—	—	—	7.15 a.m.	—
Temperature Reactions			N	P.I.N.	R	R	R	N	N
Haemoglobin gm. %			9·13	9·32	8·88	10·35	9·50	9·32	9·42
Sugar mgm. %		L	56·82	52·91	62·50	58·82	75·19	63·69	52·25
		U	50·50	47·52	56·50	48·54	68·03	57·14	—
T.N. gm. %			2·195	2·206	2·150	2·304	2·164	2·024	2·052
N.P.N. mgm. %		L	14·42	14·42	14·28	21·42	20·54	17·11	15·65
		U	13·04	12·50	10·71	17·14	15·31	13·16	—
Coag. N. gm. N %		L	2·181	2·192	2·136	2·283	2·143	2·007	2·036
		U	2·182	2·194	2·139	2·289	2·149	2·011	—
Urea	mgm. N %	L	3·35	3·81	4·26	10·77	8·75	4·43	4·26
	,, U %		7·14	4·98	9·03	22·68	18·48	9·24	9·03
	mgm. N %	U	2·98	7·98	9·03	10·56	8·30	4·13	—
	,, U %		6·30	7·56	8·19	22·26	17·43	8·61	—
Total Creatinine	mgm. N %	L	2·17	2·35	2·04	2·66	2·10	2·10	2·04
	,, TC %		5·84	6·36	5·50	7·20	5·68	5·68	5·50
	mgm. N %	U	2·01	2·04	1·75	2·27	1·82	1·82	—
	,, TC %		5·40	5·50	4·70	6·16	4·90	4·90	—
Uric acid	mgm. N %	L	0·19	0·25	0·21	0·17	0·24	0·22	0·14
	,, UA %		0·56	0·74	0·62	0·52	0·73	0·65	0·42
	mgm. N %	U	0·22	0·26	0·13	0·07	0·16	0·16	—
	,, UA %		0·67	0·62	0·40	0·22	0·48	0·48	—
Amino acid mgm. N %		L	5·51	5·00	4·67	5·22	5·38	6·67	5·79
		U	4·24	3·84	3·50	3·15	3·50	4·52	—
R.N. mgm. N %		L	3·20	3·01	3·10	2 60	4·07	3·69	3·42
		U	3·58	2·82	1·38	1·09	1·53	2·53	—
Plasma			n.u.	n.u.	n.u.	n.u.	n.u.	n.u	n.u.

Main Features of Analytical Data.

Sugar.—Increases from about 52–75 mgm. % and from 50–68 mgm. % for "laked" and "unlaked" filtrate, respectively.
N.P.N.—Increases from 14–21 and from 13–15 at the acme of the reaction.
U.N.—Increases from 3–10·5 mgm. N %.
Hb., U.A.N., T.C.N., A.A.N., R.N., and *T.N.*—Nothing unusual.

SUMMARY OF ANALYTICAL DATA FROM THE PATHOLOGICAL ASPECT.

In the nine cases of bluetongue examined, Case I represented the severest reaction, the other eight less severe, and Case IV the least, although the strain used must be considered as virulent. It was obtained from Natal from an outbreak of bluetongue in which mortalities were reported and was responsible for several deaths when subinoculated into sheep here at Onderstepoort. The conditions under which the experimental animals were kept, i.e., regular feeding, watering and shelter, are probably responsible for the absence of more severe reactions and mortalities. The severe temperature reactions noted indicate in some measure the virulency of the strain.

If the analytical data are reviewed as a whole, the following emerges :—

Haemoglobin.

In only two cases (I and II) can any definite change be established, e.g., in Case I the Hb. drops from 15·7–8·5 gm. per cent., with a relatively slow recovery, and in Case II from 15·5–12·9 gm. per cent., the decrease in both cases coinciding with the hyperthermic reaction. It should be noted that these two cases represent the more severe reactions. In the other seven cases no distinct changes in either direction are noticeable.

Sugar.

An increase is noted in all except two cases (II and IV), viz., Case I from 45–117·6 mgm. per cent.; Case III, 45–71 mgm. per cent.; Case V, 45–60 mgm. per cent.; Case VI up to 75 mgm. per cent., etc., in "laked" and correspondingly in "unlaked" filtrates. The increase may be very striking, as in Case I, or merely an increase to just over the upper limits of normal, depending on the severity of the reaction.

Non-Protein Nitrogen.

Shows an increase in all cases except Case IV, varying from ± 18–66 mgm. N per cent. in Case I, from 15–26 mgm. N per cent. in Case II, from 17–23 mgm. N per cent. in Case V, from 16–23 mgm. N per cent. in Case VII, and from 14–21 mgm. N per cent. in Case IX, with smaller increases in the other cases. The concentration in the "unlaked" filtrates rises correspondingly.

Urea Nitrogen.

Shows increase in all cases except Case IV, e.g., Case I from 5–49 mgm. N per cent., Case II 3·5–13 mgm. N per cent., Case V 4·5–11 mgm. N per cent., Case VII 4–10·4 mgm. N per cent., and Case IX from 3–10·5 mgm. N per cent. The increase in U.N. is solely responsible for the rise in the N.P.N. fraction recorded above.

Total Creatinine Nitrogen.

Except for a slight rise recorded in the severe reaction in Case I, no variations outside the normal range are noticeable.

Total Nitrogen.

This remains unchanged except in Cases I and II, i.e., cases associated with a decrease in the Hb. level of the blood.

Amino-Acid Nitrogen, Uric Acid Nitrogen and *Rest Nitrogen.*

No appreciable alterations occur.

CONCLUSION.

In bluetongue of sheep there is generally an increase in the sugar, N.P.N. and U.N. fraction of the blood. In severe cases a decrease in Hb. and a corresponding decrease in T.N. is recorded. The degree of the variations correspond approximately to the severity of the reaction.

III.—GENERAL SUMMARY.

(1) The total nitrogen and haemoglobin of whole blood, and the sugar, urea, amino-acid, " total " creatinine, uric acid and non-protein nitrogen of " laked " and " unlaked " blood filtrates of sheep have been determined.

(2) Determinations were made during the pre-infection period (" normal "), the incubation period, the actual hyperthermic reaction stage, and in recovery cases during the convalescent stage, until the level of the blood constituents had returned to normal.

(3) " Normal " data in respect of the above constituents for both " laked " and " unlaked " filtrates have been separately detailed.

(4) As far as differences in the two types of filtrates are concerned, the concentrations in " laked " filtrates are in all cases higher than in the " unlaked," the difference being least in the case of urea.

(5) Temperature charts to show the nature and type of the reaction, as well as the periods at which blood was withdrawn, have been incorporated.

(6) Well marked changes are recorded in Heartwater and Bluetongue, the degree of the change corresponding approximately to the severity of individual reactions.

ACKNOWLEDGMENTS.

In conclusion, I wish to express my thanks to Mr. W. O. Neitz for his ready co-operation in permitting me at all times to bleed his experimental animals and also for access to his records; to Mr. W. F. Averre and his assistants for bleeding the animals whenever required.

REFERENCES.

In addition to the publications referred to in the text a few additional references referring more particularly to the clinical and pathological aspect have been included, since these aspects were only very briefly dealt with.

In spite of a very extensive search no references bearing directly on the conditions investigated here could be found.

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Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 1, Number 1, 1933.

Chemical Blood Studies.*

IV. Comparative Studies on "Laked" and "Unlaked" Blood Filtrates of Horses in Health and during Horse-sickness *(Pestis equorum)*.

By H. GRAF, B.Sc., D.V.Sc., Veterinary Research Officer, Department of Chemical Pathology, Onderstepoort.

CONTENTS.

I. INTRODUCTORY NOTE.

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- (*d*) Normal Range of Values for haemoglobin, sugar, non-protein nitrogen, urea, "total" creatinine, uric acid, amino-acid, total nitrogen and rest nitrogen.
- (*e*) Summary of Range of Values during Horse-sickness.
- (*f*) Conclusion.

III. REFERENCES.

I.—INTRODUCTORY NOTE.

The present paper is one of a series of similar researches into various diseases of animals. In order to avoid needless repetition, the aims and objects of these studies, the chemical methods and technique employed and the arrangement of the data have been collected and fully discussed in the first paper (see under "References" for a list of articles published up to now in this series).

II.—HORSE-SICKNESS (*Pestis equorum*).

(*a*) SYMPTOMATOLOGY AND PATHOLOGICAL ANATOMY.

Although it is not intended to here give a full description of this disease, it may be of interest briefly to state the symptoms and post-mortem findings, more particularly with a view to correlating abnormal blood conditions, if any, with the symptomatology or the pathological anatomical diagnosis.

For fuller details the bibliography at the end of this article should be consulted. No reference in the available literature to any purely biochemical researches with horse-sickness blood could be found; physico-chemical by Frei (1907) and blood morphological researches by Neser (1923 and 1926) constitute the only investigations into the blood of equines during an attack of horse-sickness.

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* "Chemical Blood Studies, I, III–V" was accepted as Thesis for the D.V.Sc. degree by the University of Pretoria, December, 1932.

Horse-sickness has been defined (Knuth and du Toit, 1921, and Theiler. 1921) as " an acute and sub-acute infectious disease of equines, caused by an ultravisible virus, and which is generally fatal. It is not directly contagious, infection most likely being spread by bloodsucking insects. Animals which have recovered are immune and no longer harbour the virus. It occurs in South and Central Africa."

The disease is readily produced in a susceptible animal by the subinoculation of blood or serum from a reacting animal.

Clinically four different forms are distinguished, viz., horse-sickness fever, the pulmonary form (Dunkop), the oedematous or cardiac form (Dikkop), and the " mixed " form.

(*a*) *Horse-sickness Fever* is primarily characterised by its typical intermittent temperature reaction which sets in usually 5–7 days after infection, reaching its acme by the 9th to 13th day p.i., the hyperthermia decreasing generally by lysis. Other symptoms, except usually a slight loss of appetite and dullness, are absent.

(*b*) The course of the *pulmonary form* is usually fulminant or peracute, with a short incubation period of 2–5 days, rarely longer. The temperature rises rapidly to 105–106°, the animals frequently succumbing at the acme of the reaction. The characteristic symptoms noted are (in addition to hyperrhexia) a very severe dyspnoea of both inspiratory and expiratory character; coughing which is as a rule followed by a discharge of often large amounts of a yellow frothy liquid, great restlessness, dilatation of the pupils, cyanosis of the mucous membranes and death. In cases of recovery, the symptoms are less acute and the temperature reaction shows a more prolonged stadium incrementi and decrementi.

(*c*) The *oedematous* or *cardiac form* (Dikkop) is characterised by (i) an incubation period of 5–7 days, the acme being reached usually on the 12–13th day p.i., succeeded by a critic or lytic descent; the duration of the fever lasting generally 8–10 days. (ii) The development of subcutaneous oedematous swellings, particularly of the head and neck, shortly after the fever acme has been passed. These swellings are tense to the touch, as a rule not painful and in cases of recovery disappear in a few days. (iii) Cardiac symptoms as evinced by the cyanotic mucous membranes, alterations in the quality and rate of the pulse, which increases to 50 to 60, becoming gradually weaker, softer and thready; arythmic, dicrotic or deficient pulses are also met with. The cardiac area of impulse increases, the heart sounds becoming weaker and more diffuse. A cooling of the extremities is often noted, especially in fatal cases. (iv) A dyspnoea, which, however, is not as pronounçed as in the Dunkop form. (v) Loss of appetite setting in at the onset of the fever reaction and going over to complete anorexia is often seen. Complications such as paralysis of the oesophagus, gangrenous pneumonia and colic may be sometimes observed.

Pathological Anatomy.

At post-mortem the following characteristic changes are observed:—

(*a*) The pulmonary (Dunkop) form shows a severe oedematous transudation into the tissues of the lungs, subpleural tissue, regional lymph glands and submucosa of the trachea with a clear greenish-yellow fluid; hydrothorax, the pleural cavity containing up to several litres of clear fluid; subepicardial and subendocardial haemorrhages; hyperaemia of the liver and kidneys—rarely associated with degenerative changes.

(*b*) The cardiac, oedematous or "Dikkop" form is characterised by transudation of the subcutical tissues with a pale yellowish fluid chiefly in the region of the head and neck, fasciae and muscular aponeuroses and regional lymph glands; cyanosis of the mucous membranes; hydropericardium—the amount of liquid varying from ½-litre to 2½-litres; extensive subepicardial and subendocardial haemorrhages; hyperaemia and fatty degeneration of liver and kidneys, degeneration of the myocardium.

Experiments with Horse-sickness.

For these analyses blood was drawn from horses which had been artificially infected by inoculation with virulent blood (except Case III) for investigations also into other aspects of the disease, particularly transmission and immunisation experiments. I here wish to place on record my sincere thanks to my colleagues Messrs. W. O. Neitz and R. du Toit, for their ready permission to bleed their experimental subjects at any time for the purpose of my analyses and for their courtesy in informing me in good time of likely suitable cases.

The horse were of various breeds and conditions, and ages, details being given under "History" in each case. The rations supplied are given in "Chemical Blood Studies" I (see this Journal).

Most of the animals were placed on temperature for months or weeks prior to infection, but in this paper only the temperature records of the last 2-3 weeks have been reproduced. The normal temperature records run a remarkably even course, showing for months on end only the slight diurnal variations, with a "mean" level at about 100°.

(*b*) METHODS OF ANALYSIS AND TECHNIQUE.

Exactly the same procedure was followed as that described in the first paper of this series ("Chemical Blood Studies I," this Journal). In connexion with the study of equine blood, particularly horse blood, the precaution of *thorough* shaking of the oxalated blood must be observed before pipetting off any blood for the preparation of filtrates, total nitrogen and haemoglobin determinations, otherwise the results will be inaccurate, because with equine blood sedimentation of the corpuscular elements is *very* rapid.

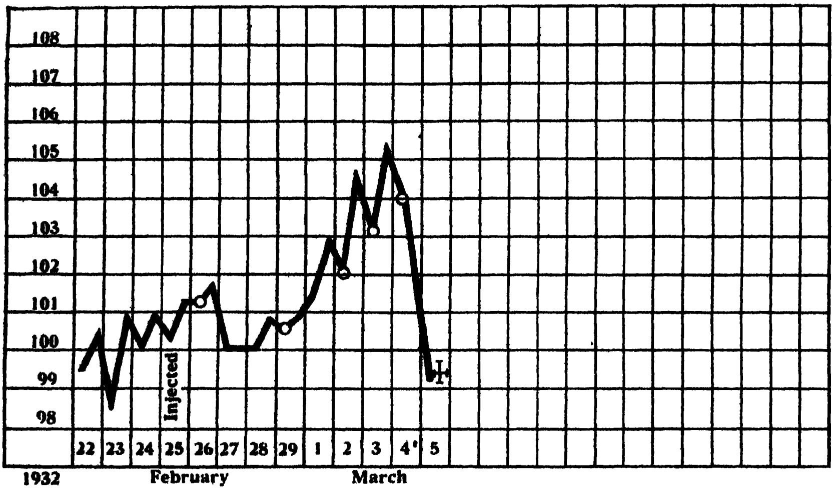
For the morphological aspect of equine blood, the red, white and differential counts, "red volume %" etc., I would refer to the works of Neser (1926) more particularly as the major part of those researches were also carried out here at Onderstepoort and the results obtained would, therefore, be more directly applicable to the bloods handled by me than if those researches had been carried out under different environmental conditions.

In presenting the experimental data of 14 horse-sickness cases the same sequence as previously will be adhered to, i.e., the temperature record (only for the actual hyperthermic period) on which small circles indicate the times of bleeding, followed by a history of the experimental subject, the analytical data and the main features emerging from the data collected. A statement of the normal values of all constituents determined will precede a general summary of the results obtained.

(c) EXPERIMENTAL DATA.

CASE I.

Horse 20302. *Horse-sickness* (*Dikkop*). *Died* 5/3/32.
Temperature Chart I.



History : Horse 20302. An aged, dark-chestnut stallion, in good condition; was placed on temperature 11/12/31, and used for mosquito feeding experiments (Messrs. Bedford and du Toit), after being injected on 25/2/32 intrajugularly with 5 c.c. blood sent in by the Government Veterinary Officer, Eshowe. On 2/3/32 100 c.c. blood was drawn and mosquitos allowed to feed on this animal. On the fourth day p.i. the temperature reaction set in, reaching 105·6° within the following 72 hours, the temperature dropping by crisis, the animal succumbing on the 9th day p.i. from "Dikkop" horse-sickness. At post-mortem there was found to be present, transudation of the subcutis and loose connective tissue, cyanosis, slight hydrothorax, hydropericard, tumor splenis, subserous haemorrhages and slight verminosis of the digestive tract.

TABLE 1.

Horse 20302.

Date *Time*		26/2/32. —	29/2/32. —	2/3/32. —	3/3/32. —	4/3/32 —
Temporary Reactions		N	P.I.R.	R	R	R
Hb. gm. %		15·42	14·49	12·83	12·42	14·90
Sugar mgm. %	L	83·3	95·2	98·0	106·4	104·2
	U	67·6	77·0	77·0	88·5	96·2
T.N. gm. %		3·269	3·101	2·863	2·912	3·038
N.P.N. mgm. %	L	21·43	19·34	18·35	19·36	19·25
	U	14·89	14·63	14·00	14·81	15·11
Coag. N. gm. N %	L	3·248	3·083	2·845	2·893	3·019
	U	3·254	3·086	2·849	2·897	3·023
Urea mgm. N %	L	6·42 13·44	7·80 16·38	5·73 11·97	7·24 15·12	7·36 15·54
	U	6·13 12·81	7·30 16·38	5·69 11·97	6·72 14·07	6·94 14·49
Total Creatinine mgm. N %	L	2·36 6·40	2·11 5·70	1·86 5·00	1·82 4·90	1·82 4·96
	U	1·86 5·00	1·78 4·80	1·60 4·30	1·45 3·90	1·93 5·20
Uric acid mgm. N %	L	0·82 2·46	0·67 2·00	0·82 2·45	0·67 2·00	0·67 2·00
	U	0·40 1·20	0·23 0·70	0·27 0·80	0·30 0·90	0·57 1·71
Amino acid mgm. N %	L	7·80	6·44	6·14	7·00	7·00
	U	4·70	3·35	4·54	4·44	4·10
R.N. mgm. N %	L	4·03	2·67	3·80	2·63	2·40
	U	1·80	1·53	1·90	1·90	1·57
Plasma		—	—	—	—	—

Main Features of Data.

Hb.—Shows a drop from 15·42 to 12·42 gm. per cent. with a subsequent rise to 14·90 gm. per cent. on day prior to death.

Sugar.—This rises from 83 mgm. per cent. to 106·4 mgm. per cent. in the case of "laked" and from 67·6–96·2 mgm. per cent. in "unlaked" filtrates.

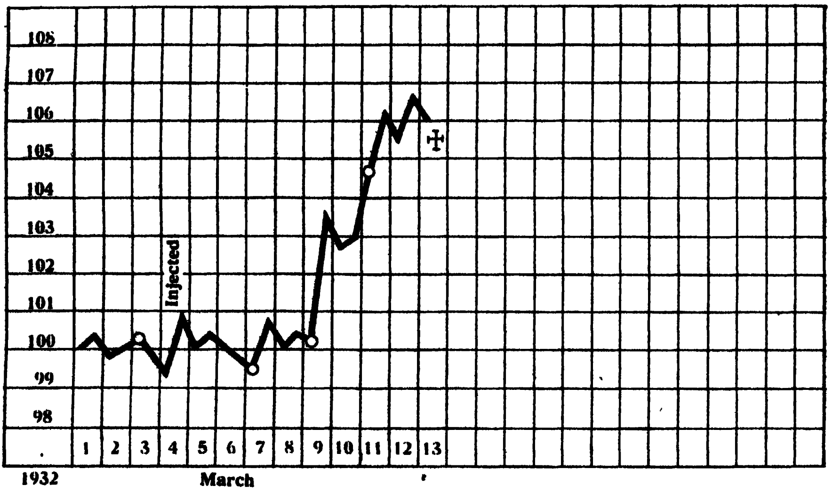
T.C.N.—A decrease from 2·36–1·82 mgm. N per cent. is noted in "laked" and from 1·86–1·45 mgm. N per cent. in "unlaked" filtrate, with a rise on day before death to 1·93 mgm. N per cent.

T.N.—A slight decrease running parallel with Hb. values.

U.P.N., *U.N.*, *U.A.N.*, *A.A.N.*, and *R.N.*—No changes in either direction are noted.

CASE II.

Horse 20262. *Horse-sickness* (*mixed type*). *Died* 13/3/32.
Temperature Chart II.



History: Horse 20262. A chestnut gelding, approximately 12 years old, in good condition. Was placed on temperature on 20/10/31, showing a regular normal temperature record. On the 4th of March this animal was injected intrajugularly with 5 c.c. blood from a mule (*ex* Agricultural School, Losperfontein). On the 5th day p.i. the temperature reaction set in reaching 106·4° within 48 hours of the onset. The animal died on 3/3/32, i.e., on the 9th day p.i. Anorexia, dyspnoea and swelling of the supra-orbital fossa were noted. The pathological anatomical findings were cyanosis of the mucous membranes, transudation of plasma into the subcutis, loose connective tissue throughout the body and subpleurally. Severe hydrothorax and oedema of the lungs. hydropericard, subendocardial haemorrhages, degeneration of the myocard, fatty degeneration and hyperaemia of the liver and kidneys and slight tumor splenis. The clinical and post-mortem findings suggest this to have been a case of the "mixed".type of horse-sickness..

Table 2.

Horse 20262.

Date		3/3/32.	7/3/32.	9/3/32.	11/3/32.
Time		—	—	—	—
Temperature Reactions		N	P.I.N.	R	R
Hb. gm. %		18·57	18·96	22·63	15·42
Sugar mgm. %	L U	64·5 58·5	71·4 62·1	57·8 47·8	100·0 83·3
T.N. gm. N %		3·437	3·465	3·465	3·031
N.P.N. mgm %	L U	22·5 16·5	27·0 17·8	27·5 17·5	21·1 15·3
Coag. N gm. N %	L U	3·415 3·420	3·438 3·447	3·438 3·448	3·010 3·016
Urea mgm. N %	L	7·30 15·33	9·00 18·90	9·50 19·95	9·90 20·79
	U	6·60 13·86	9·00 18·90	9·00 18·90	9·40 19·74
Total Creatinine mgm. N %	L	2·01 5·40	2·01 5·40	2·19 5·84	2·29 6·20
	U	2·10 5·70	1·90 5·10	2·10 5·68	1·50 4·00
Uric acid mgm. N %	L	0·53 1·60	0·53 1·60	0·58 1·73	0·50 1·50
	U	0·53 1·60	0·30 0·89	0·47 1·40	0·20 0·60
Amino acid mgm. N %	L	10·8 5·8	12·7 3·9	8·7 4·5	8·2 3·7
R.N. mgm. N %	L U	1·90 1·50	2·80 2·72	6·50 1·40	1·85 0·50
Plasma		—	—	—	—

Salient Features of Analytical Data.

Hb.—Shows a rise from 18·57–22·63 gm. per cent., succeeded by a drop to 15·42 gm. per cent. 48 hours before death.

Sugar.— An increase is towards the end noted (±66–100 mgm. per cent.).

T.N.—A decrease is noted coinciding with the drop of Hb. value.

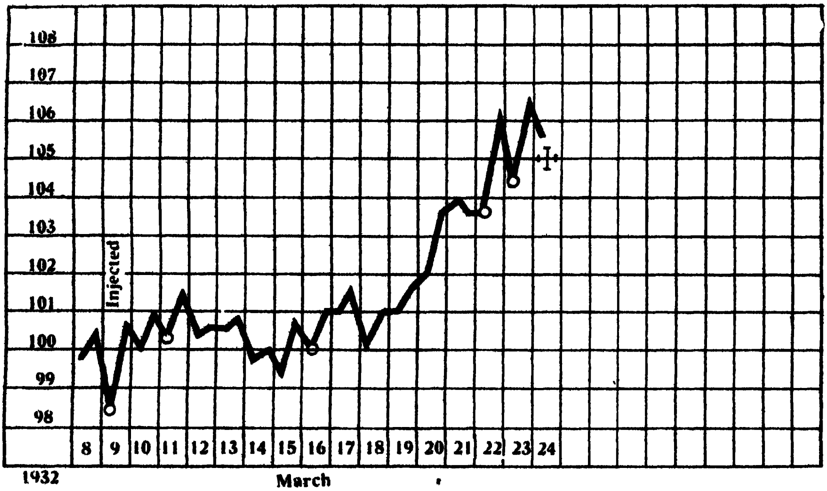
N.P.N.—A tendency towards a slight increase in the incubation period is succeeded by a drop 48 hours before death. The changes are, however, only within the normal variation.

U.N., T.C.N., U.A.N., R.N.—Nothing unusual.

A.A.N.—Shows a slight decrease (anorexia ?).

CASE III.

Horse 20288. *Horse-sickness* (*mixed*). *Died* 24/3/32.
Temperature Chart III.



History: Horse 20288. Bay gelding, aged, in medium condition, was placed on temperature 11/12/31 (mosquito transmission experiments, Messrs. Bedford and du Toit). The temperature remained normal until 19/3/32, when a temperature reaction set in, the horse dying six days later from horse-sickness. This case is of interest in that horse-sickness was apparently produced through the subcutaneous injection of mosquitos on the 9th, 10th and 11th March, 1932. These mosquito transmission experiments will be dealt with by their authors (Messrs. Bedford and du Toit) in the next report of the Director of Veterinary Services, to which those interested are referred. The post-mortem findings substantiate the clinical diagnosis of horse-sickness. the pathological findings being transudation into subcutis, subpleural and loose connective tissue, slight hydropericard, extensive subendocardial haemorrhages, oedema of the lungs, hyperaemia and fatty degeneration of the liver and slight tumor splenis.

TABLE 3.

Horse 20288.

Date *Time*		9/3/32. —	11/3/32. —	16/3/32. —	22/3/32. —	23/3/32. —
Temperature Reactions		N	N	P.1.N.	R	R
Hb. gm. %		17·84	16·87	15·19	13·31	13·31
Sugar mgm. %	L	70·92	74·60	71·43	105·36	93·46
	U	57·50	54·30	58·48	91·76	87·72
T.N. gm. %		3·220	3·465	3·325	2·874	3·094
N.P.N. mgm. %	L	23·10	19·03	18·75	18·75	21·23
	U	17·80	15·00	15·00	15·15	16·86
Coag. N gm. N %	L	3·197	3·446	3·306	2·795	3·073
	U	3·202	3·450	3·310	2·799	3·077
Urea mgm. N %	L	10·70 22·47	8·53 17·85	6·57 13·86	7·00 14·70	8·09 17·01
	U	10·30 21·63	8·91 18·69	6·63 13·86	7·16 15·12	8·09 17·01
Total Creatinine mgm. N %	L	2·40 6·40	1·86 5·00	2·04 5·50	1·84 4·96	1·98 5·32
	U	1·90 5·14	1·41 3·70	1·58 4·24	1·56 4·22	1·90 5·14
Uric acid mgm. N %	L	0·71 2·14	0·64 1·91	0·63 1·89	0·44 1·31	0·44 1·31
	U	0·70 2·10	0·20 0·60	0·30 0·91	0·39 1·16	0·36 1·07
Amino acid mgm. N %	L	7·39	6·90	7·37	6·51	7·00
	U	4·10	3·50	4·00	3·50	3·50
R.N. mgm. N %	L	1·90	1·06	2·14	2·96	3·72
	U	0·80	1·98	2·49	2·54	3·00
Plasma		—	—	—	—	—

Main Features of Analytical Data.

Hb.—Drops from 17·84–13·31 gm. per cent.

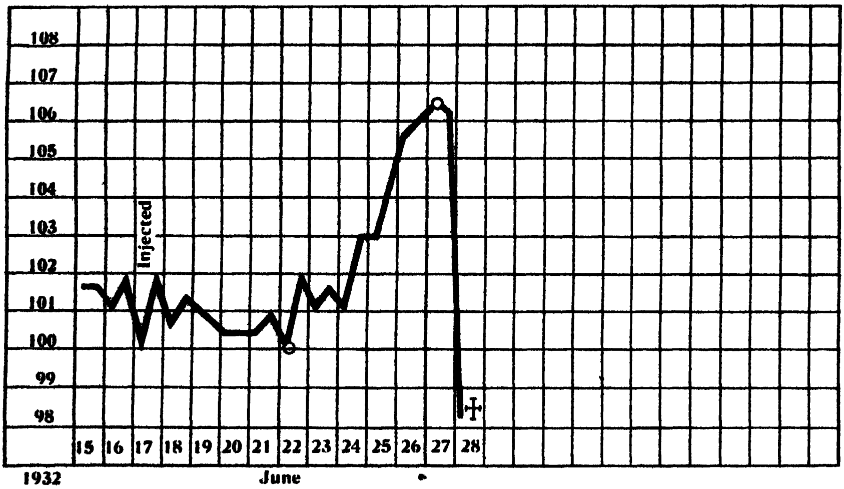
Sugar.—Increases from 70·92–105·36 mgm. per cent. and drops 24 hours before death to 93·46 mgm. per cent.

T.N.—Tending to drop slightly.

N.P.N., U.N., T.C.N., U.A.N., A.A.N. and ***R.N.***—Show no marked or definite alterations but in all there is a *slight* tendency towards a decrease.

CASE IV.

Horse 20276. *Horse-sickness* (*Dunkop*). *Died* 28/6/32.
Temperature Chart IV.



History: Horse 20276. A light bay gelding, 12 years old, in poor condition. Was placed on temperature 23/12/31 and utilised in mosquito transmission experiments (Messrs. Bedford and du Toit). The temperature remained normal throughout. The horse was injected on 17/6/32 intrajugularly with 2 c.c. blood from mule 6396 (*ex* Agricultural Training School, Losperfontein). The temperature reaction set in on the 7th day p.i. the horse succumbing three days later. On the 27th June the horse received 1 gm. Acriflavin (Mr. Parkin) shortly *after* the blood was drawn for analyses. At the post-mortem examination there was found to be present the characteristic pathological changes associated with horse-sickness, e.g., transudation into subcutis and loose connective tissue, hydropericard, marked oedema of the lungs, a slight fatty degeneration change of the liver and pigmentation of the renal cortex.

Date / *Time*		15/4/32. 11 a.m.	26/4/32. —	3/6/32. 7.15 a.m.	6/6/32. —	9/6/32. —	14/6/32. —	22/6/32. —	27/6/32. —
Temperature Reaction		N	N	N	N	N	N	P.I.N.	R
Hb. gm. %		14·72	14·08	17·51	13·31	14·72	15·19	15·42	19·33
Sugar mgm. %	L	89·28	97·74	66·67	85·47	83·33	—	75·75	60·98
	U	80·65	83·83	57·14	76·34	73·56	—	66·67	55·55
T.N. gm. %		3·172	2·976	3·242	2·780	2·892	2·990	3·004	3·291
N.P.N. mgm. %	L	23·08	24·04	19·36	19·37	18·65	18·75	20·98	20·00
	U	18·50	18·61	14·56	13·82	14·28	14·32	15·87	17·65
Coag. N gm. N %	L	3·149	2·952	3·223	2·761	2·873	2·971	2·983	3·271
	U	3·154	2·957	3·227	2·767	2·878	2·976	2·988	3·273
Urea mgm. N %	L	9·25	9·87	6·49	6·02	7·00	6·24	7·70	7·44
		19·53	20·79	13·65	12·60	14·70	13·02	16·17	15·54
	U	8·52	8·90	6·35	5·66	6·50	5·45	7·40	7·83
		17·85	18·69	13·44	11·97	13·65	11·55	15·54	16·38
Total Creatinine mgm. N %	L	2·42	2·42	2·42	2·01	2·23	—	2·50	2·76
		6·54	6·54	6·54	5·40	6·00	—	6·74	7·41
	U	2·23	2·23	2·23	2·23	2·10	—	2·23	2·66
		6·00	6·00	6·00	6·00	5·68	—	6·00	7·20
Uric acid mgm. N %	L	0·60	0·24	0·23	0·30	0·30	—	0·27	0·29
		1·80	0·73	0·69	0·69	0·91	—	0·80	0·87
	U	0·43	0·21	0·18	0·25	0·23	—	0·16	0·32
		1·28	0·62	0·55	0·75	0·69	—	0·48	0·97
Amino acid mgm. N %	L	7·78	8·24	5·83	6·67	6·33	7·45	6·19	7·14
	U	4·83	4·52	4·00	3·59	3·67	4·95	4·62	5·00
R.N. mgm. N %	L	3·03	3·33	4·39	4·44	2·79	5·06*	4·32	2·37
	U	2·50	2·75	1·86	2·09	1·78	3·97*	1·46	1·84
Plasma		n.u.	n.u.	n.u.	n.u.	n.u.	n.u.	n.u.	Slight orange

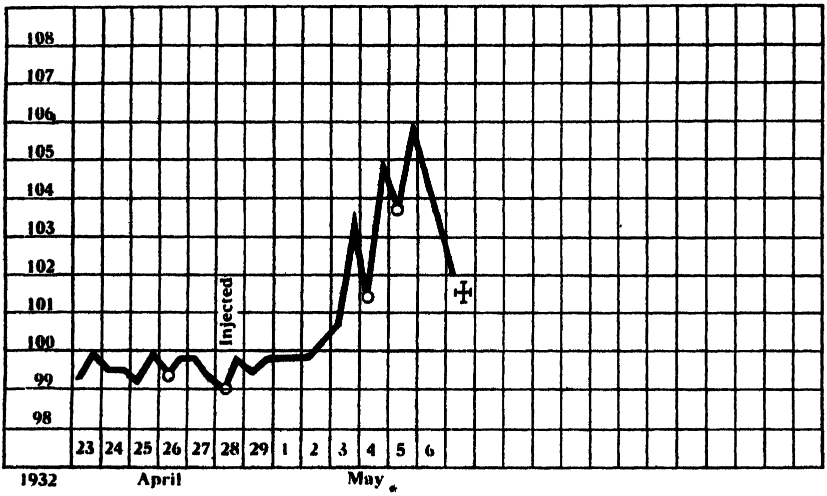
* Includes "Total Creatinine N" and "Uric Acid N."

Main Features of Analytical Data.

Hb.—An increased Hb. content at the height of the reaction a day before death is noted.
Sugar.—The lowest figure is recorded at height of reaction (for both filtrates).
T.N., *N.P.N.*, *U.N.*, *T.A.N.*, and *R.N.*—Nothing specific—variations within normal range.
T.C.N.—Shows an increase from about 2·30–2·76 mgm. N per cent. in "laked" and from 2·15–2·66 mgm. N per cent. in "unlaked" filtrate.

CASE V.

Horse 20130. *Horse-sickness* (*Dikkop*). *Died* 6/5/32.
Temperature Chart V.



History: Horse 20130. Bay gelding, 8 years old and in medium condition. Was placed on temperature 7/8/31 and used in mosquito transmission experiments (Messrs. Bedford and du Toit). Except for a slight, indefinite temperature reaction during the 6th to the 10th April, 1932, the temperature record was normal. On the 28th April, 1932, 1 c.c. blood from Horse 20302, Eschowe strain (*vide*) was injected subcutaneously. The temperature reaction set in on the fourth day p.i. The symptoms shown were those of a "Dikkop" horse-sickness reaction.

At the post-mortem examination (P.M. No. 11033 of 6/5/32) there was found a generalised cyanosis, transudation into the subcutis and loose connective tissue, hydrothorax, hydropericard, subepicardial and subendocardial haemorrhages, degeneration of the myocard, hyperaemia and slight fatty degeneration of the liver.

TABLE 5.

		14/3/32.	17/3/32.	23/3/32.	12/4/32.	26/4/32.	28/4/32.	3/5/32.	4/5/32.	6/5/32.*
Date		14/3/32.	17/3/32.	23/3/32.	12/4/32.	26/4/32.	28/4/32.	3/5/32.	4/5/32.	6/5/32.*
Time		—	—	—	—	—	—	—	—	—
Temperature Reactions		N	N	N	N	N	N	R	R	R
Hb. gm. %		15·42	17·84	15·42	18·20	20·18	15·42	14·84	13·87	25·24
Sugar mgm. %	L	82·60	101·00	93·46	57·47	119·04	94·34	117·64	161·30	129·88
	U	66·20	80·65	89·30	—	84·03	71·94	105·26	142·86	103·10
T.N. gm. %		3·374	3·388	2·968	3·654	3·535	3·290	2·968	2·912	4·060
N.P.N. mgm. %	L	24·40	25·27	26·64	26·08	27·77	26·08	25·00	18·75	56·54
	U	18·70	18·20	17·54	18·75	20·08	17·85	18·61	16·66	47·62
Coag. N gm. N %	L	3·350	3·363	2·942	3·628	3·508	3·264	2·943	2·893	4·003
	U	3·355	3·370	2·950	3·635	3·515	3·272	2·949	2·895	4·012
Urea mgm. N %	L	12·30	9·20	10·11	10·11	12·33	10·43	10·48	8·90	21·08
„ U %		25·83	19·32	21·21	21·21	25·83	21·84	22·05	18·69	44·31
mgm. N %	U	11·50	8·52	9·53	9·00	11·34	9·58	10·11	8·80	20·40
„ U %		24·15	17·85	19·95	18·90	23·73	20·16	21·21	18·48	42·84
Total Creatinine mgm. N %	L	2·40	2·01	2·23	2·33	2·23	1·89	2·23	1·97	4·91
„ TC %		6·60	5·40	6·00	6·26	6·00	5·14	6·00	5·32	13·48
mgm. N %	U	2·00	1·75	1·89	2·04	2·23	1·49	1·78	1·41	—
„ TC %		5·40	4·70	5·14	5·54	6·00	4·00	4·80	3·80	—
Uric acid mgm. N %	L	0·30	0·39	0·27	0·43	0·29	0·41	0·28	0·25	0·63
„ UA %		0·84	1·16	0·81	1·32	0·87	1·22	0·84	0·74	1·88
mgm. N %	U	0·13	0·19	0·20	—	0·18	0·27	0·19	0·18	—
„ UA %		0·38	0·58	0·61	—	0·53	0·80	0·58	0·53	—
Amino acid mgm. N %	L	7·40	7·78	7·37	6·86	8·75	7·61	8·75	6·36	14·00
	U	3·70	4·52	3·68	3·89	4·12	4·12	3·89	4·12	11·66
R.N. mgm. N %	L	2·00	5·89	6·66	5·35	4·19	5·74	4·26	1·47	14·92
	U	1·51	3·22	2·24	3·00†	2·21	2·39	3·74	2·15	15·56‡
Plasma		—	—	—	—	—	—	—	—	—

* Animal down—blood drawn about four hours before death. † Includes "Uric Acid N." ‡ Includes "Uric Acid N" and "Creatinine N."

Main Features of Analytical Data.

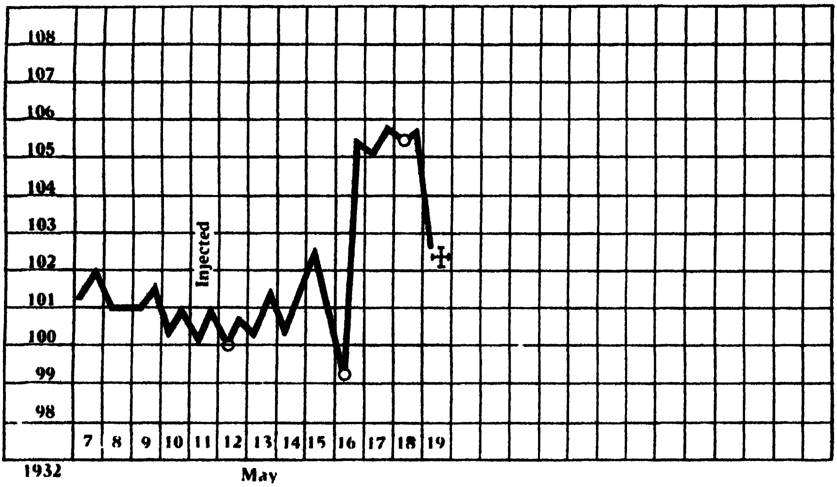
Hb.—High Hb. content in blood drawn on last day four hours before death. The animal was down and already in agonal stage.

Sugar.—Shows a steady rise in the "laked" filtrate to 161·30 mgm. per cent. the day before death, with decrease to 129·88 mgm. per cent. four hours before death; similar changes in "unlaked" filtrate.

T.N., *N.P.N.*, *U.N.*, *T.C.N.*, *U.A.N.*, *A.A.N.*, and *R.N.*—All these constituents show no changes—outside the limits of normal variation—except in the blood drawn on 6/5/32 four hours before death, in which they are all greatly increased in amount, in most cases more than doubled.

CASE VI.

Horse 20300. *Horse-sickness* (*Dunkop*). *Died* 19/5/32.
Temperature Chart VI.



History: Horse 20300. A dark chestnut mare, 7 years old, in fair condition. Was placed on temperature on 21/3/32, being used for mosquito transmission experiments (Messrs. Bedford and du Toit). On the 11th of May, 1932, this animal received 5 c.c. blood intrajugularly from horse 20288 (vide). On the fifth day p.i. the temperature reaction set in, rising to 105·2° within 12 hours, the animal succumbing three days later from Dunkop horse-sickness. The post-mortem findings were cyanosis of the mucous membranes, oedema and hyperaemia of the lungs, hydropericard, subepicardial and subendocardial haemorrhages, general passive hyperaemia of all the organs, ascaris and habronema infection.

Horse 20300.

TABLE 6.

Date		18/4/32.	19/4/32.	22/4/32.	25/4/32.	27/4/32.	6/5/32.	12/5/32.	16/5/32.	18/5/32.
Time		—	—	—	—	—	—	—	—	—
Temperature Reaction		N	N	N	N	N	N	P.I.N.	R	R
Hb. gm. %		14·95	15·42	16·66	14·95	15·98	17·18	19·33	19·42	16·87
Sugar mgm. %	L	96·15	—	84·74	102 04	97·09	84·03	102·04	94·34	99·91
	U	71·43	—	66·67	86·96	71·43	80·00	69·93	73·56	67·11
Total Nitrogen gm. %		2·808	3·116	3 032	2·920	3·200	3·354	3·354	3·179	2·934
N.P.N. mgm. %	L	19 36	22·94	20 54	20·83	20·68	21·14	21·43	18·75	20·00
	U	15·00	15·28	15·30	13·63	14·92	15·00	15·28	13·63	14·62
Coag. N gm. %	L	2·789	3·094	3·011	2·899	3·179	3·333	3·333	3·160	2·914
	U	2 793	3·101	3·017	2·906	3·185	3·339	3·339	3·165	2·919
Urea mgm. N %	L	5·89	7·33	6·27	4·70	7·00	6·46	6·84	6·02	6·63
,, U %		12·39	15·33	13·23	9·87	14·70	13·65	14·28	12·60	13·86
mgm. N %	U	5·78	6 69	6·13	4·40	6·69	6·12	6·40	5·66	6·40
,, U %		12·18	14·07	12·81	9·24	14·07	12·81	13·44	11·97	13·44
Total Creatinine mgm. U %	L	2·06	1·91	1 67	1·52	1·91	1·98	2·04	2·01	1·82
,, TC %		5·54	5·14	4 50	4·10	5·14	5·32	5·50	5·40	4·90
mgm. N %	U	1·40	1·26	1·33	1·01	1·49	1·61	1·49	1·51	1·45
,, TC %		3·78	3·40	3·60	2·66	4·00	4·32	4·00	4·08	3·92
Uric acid mgm. N %	L	0·41	0·44	0·41	0·41	0·58	0·48	0·51	0·43	0·50
,, UA %		1 22	1 31	1·22	1·22	1·73	1·45	1·52	1·29	1·50
mgm. N %	U	0·31	0·37	0·31	0·18	0·42	0 37	0 20	0·32	0·22
,, UA %		0·94	1 10	0·94	0·55	1·25	1·12	0·61	0·97	0·66
Amino acid mgm. N %	L	8·24	9·33	9·79	8·00	8·75	8·24	8·75	7·49	8·24
	U	4·67	4·52	4·38	4·38	3·89	5·83	5·00	4·00	3·89
R.N. mgm. N %	L	2·78	3·93	2·40	6·20	2 44	3·99	3·29	2·80	2·81
	U	2·84	2·44	3·15	3·66	2·53	1·07	2·18	2·14	1·66
Plasma		n.u.	n.u.	n.u.	n.u.	n.u.	n.u.	n.u.	n.u.	Pale orange

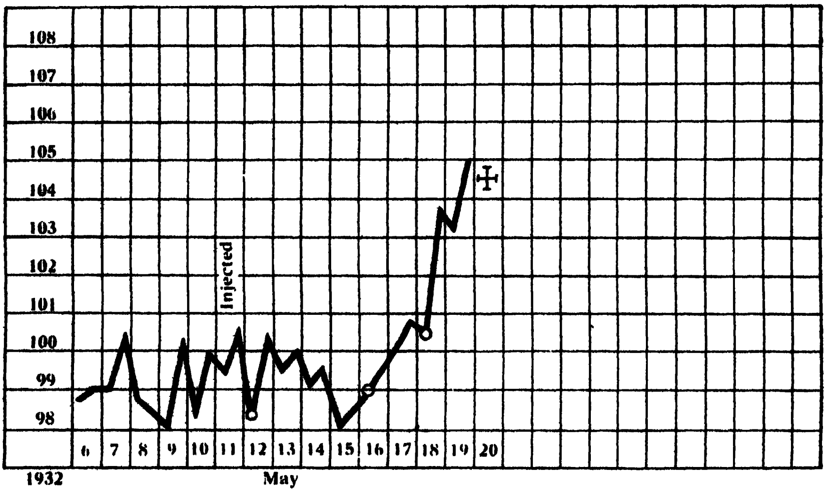
Main Features of Analytical Data.

Hb.—Variable, but highest Hb. content is during part of incubation period and at initial stage of the temperature reaction, dropping slightly on day before death.

Sugar, T.N., N.P.N., U.N., T.C.N., U.A.N., A.A.N., and *R.N.*—Nothing abnormal noted.

CASE VII.

Horse 20297. *Horse-sickness* (*Dikkop*). *Died* 20/5/32.
Temperature Chart VII.



History: Horse 20297. 14 year old bay gelding, fairly poor condition, was placed on temperature on 11/12/31 and used for various mosquito transmission experiments (Messrs. du Toit and Bedford). The temperature remained normal throughout. On 11/5/32 the horse was injected with 5 c.c. blood from mule 6396. The temperature reaction set in on the fifth day, reaching 105° on the eighth day p.i., the animal succumbing at about 10 a.m. on 20/5/32. The patient showed typical dikkop horse-sickness symptoms. The pathological anatomical findings included a slight general anaemia, numerous subendocardial haemorrhages, hyperaemia of the lungs, marked degeneration of the liver, slight strongylosis, and a heavy infection with trichonema, slight gastritis and enteritis catarrhalis.

TABLE 7.

Horse 20297.

Date............ *Time*............		15/4/32. 11 a.m.	27/4/32. —	12/5/32. —	16/5/32. —	18/5/32. —	20/5/32. *
Temp. R.........		N	N	P.I.N.	P.I.N.	R	R
Hb. gm. %.......		11·67	13·68	13·68	18·11	14·28	23·18
Sugar mgm. %	L	75·19	111·10	105·23	94·34	80·65	77·52
	U	—	95·24	91·75	79·36	73·56	55·87
T.N. gm. %......		2·794	2·738	2·710	3·025	2·696	—
N.P.N. mgm. %	L	27·27	25·00	23·71	23·08	21·43	33·72
	U	—	19·75	16·48	18·41	15·00	25·74
Coag. N. gm. N %	L	2·767	2·713	2·686	3·002	2·675	—
	U	--	2·718	2·694	3·007	2·681	—
Urea mgm. N %	L	13·29 27·93	11·65 24·57	8·90 18·69	10·83 22·68	8·17 17·22	12·42 26·04
	U	— —	12·51 26·25	8·56 18·06	10·11 21·21	7·47 15·75	12·16 25·62
Total Creatinine mgm. N %	L	2·80 7·58	2·42 6·54	2·23 6·00	2·42 6·54	2·27 6·16	2·42 6·54
	U	— —	1·82 4·90	1·75 4·70	2·01 5·40	1·61 4·32	2·04 5·50
Uric acid mgm. N %	L	0·70 2·09	0·44 1·31	0·37 1·10	0·36 1·07	0·30 0·89	0·58 1·75
	U	— —	0·38 1·14	0·21 0·64	0·30 0·91	0·27 0·82	0·34 1·02
Amino acid mgm. N %	L	7·37	8·24	7·00	7·00	6·36	8·00
	U	—	4·38	5·04	3·89	3·68	5·04
R.N. mgm. N %	L	3·11	1·39	5·21	2·47	4·33	10·30
	U	—	1·54	0·92	2·10	1·97	6·16
Plasma..........		n.u.	n.u.	n.u.	slightly orange	orange	orange

* Drawn one hour before death.

Main Features of Analytical Data.

Hb.—Shows normal variation, except on day of death when the Hb. value has become increased to 23·18 gm. per cent.

Sugar.—No change.

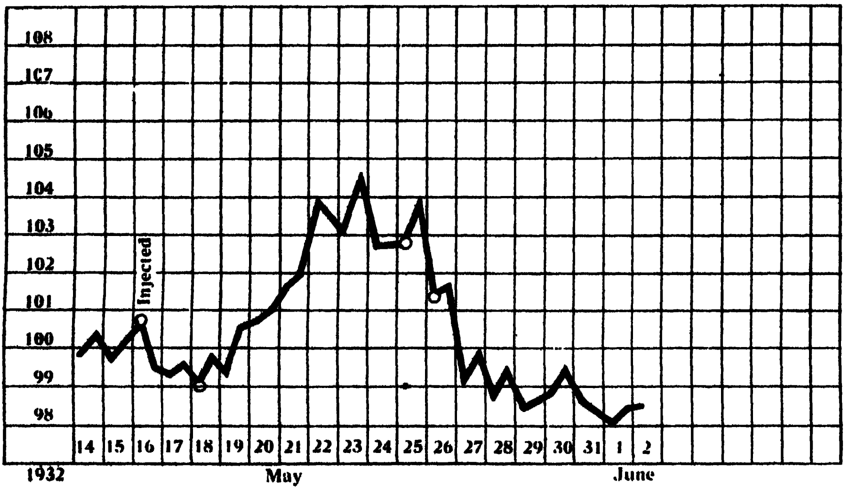
N.P.N.—Except for a slight increase in the blood drawn shortly before death, no definite changes are noticeable.

R.N.—This N fraction is markedly increased just before death.

U.N., *T.C.N.*, *U.A.N.*, *A.A.N.* and *T.N.*—Show no alterations.

CASE VIII.

Horse 20308. *Horse-sickness* (*Recovered*), *May/June*, 1932.
Temperature Chart VIII.



History: Horse 20308. Dark brown gelding, about 8 years old, in fair condition. Was placed on temperature 14/3/32 and used for various immunisation experiments (Mr. Alexander). On 16/5/32 it received intrajugularly 5 c.c. blood from Horse 20065 (O. virus 191st generation). The temperature reaction set in after four days, lasting for eight days, when the temperature had returned to normal. The horse did not show marked clinical symptoms, only a light anorexia lasting for two to three days. After recovering, the animal was kept on temperature and is still under observation at the moment.

Horse 20308.

TABLE 8.

		12/5/32.	13/5/32.	16/5/32.	18/5/32.	25/5/32.	26/5/32.	3/6/32.
Date *Time*		12/5/32. —	13/5/32. —	16/5/32. —	18/5/32. —	25/5/32. —	26/5/32. —	3/6/32. —
Temperature Reaction		N	N	N	P.I.N.	R	R	N
Hb. gm. %		16·87	16·87	14·72	15·19	12·94	11·33	15·19
Sugar mgm. %	L	81·97	73·00	74·63	75·19	75·19	73·00	71·94
	U	66·23	59·52	59·52	64·94	61·35	65·79	62·11
T.N. gm. %		3·130	3·186	3·102	3·200	2·710	2·598	3·018
N.P.N. mgm. %	L	22·06	27·27	24·62	22·72	18·93	17·65	21·06
	U	16·30	21·43	18·65	16·66	14·42	14·28	14·56
Coag. N gm. N %	L	3·108	3·159	3·077	3·177	2·691	2·580	2·997
	U	3·114	3·165	3·083	3·183	2·696	2·584	3·003
Urea mgm. N %	L	9·25 19·53	11·58 24·15	10·83 22·68	8·85 18·69	6·84 14·28	7·33 15·33	6·17 12·81
	U	9·00 18·90	10·90 22·89	10·77 22·68	8·43 17·64	6·75 14·07	6·84 14·28	5·76 12·18
Total Creatinine mgm. N %	L	2·10 5·68	1·87 5·02	2·50 6·74	2·27 6·16	2·10 5·68	2·23 6·00	1·97 5·32
	U	1·58 4·24	1·61 4·32	1·82 4·90	1·90 5·14	1·61 4·32	1·87 5·02	1·54 4·16
Uric acid mgm. N %	L	0·25 0·76	0·25 0·76	0·24 0·74	0·28 0·85	0·25 0·76	0·21 0·62	0·21 0·64
	U	0·18 0·53	0·24 0·71	0·17 0·51	0·17 0·51	0·15 0·46	0·15 0·44	0·12 0·36
Amino acid mgm. N %	L	7·00	8·33	7·78	8·43	7·00	5·83	6·09
	U	4·00	4·24	4·12	4·00	3·68	3·50	3·78
R.N. mgm. N %	L	3·56	5·24	3·26	2·69	2·74	2·05	6·52
	U	1·54	4·44	1·77	2·19	2·23	1·93	4·36
Plasma		n.u.	n.u.	n.u.	Orange	Slight orange	Pale orange	n.u.

Main Features of Analytical Data.

Hb.—The lowest Hb. values are found during the actual reaction rising to normal shortly after the return of temperature to normal.

U.N.—Shows a slight tendency to decrease.

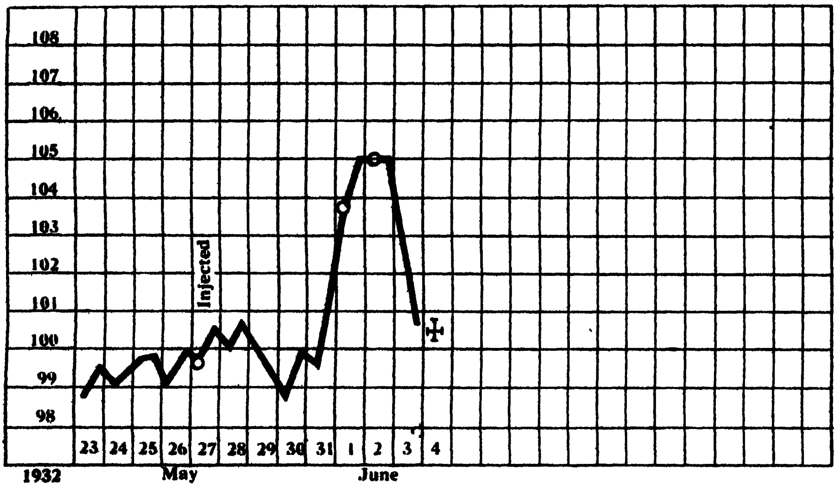
A.A.N.—Shows a slight tendency to decrease.

R.N.—This is highest shortly after return of temperature to normal, but the amount of N. is within the normal range and this finding is probably merely coincidental.

N.P.N., *Sugar*, *T.C.N.*, *U.A.N.*, and *T.N.*—Show no change in either direction.

CASE IX.

Horse 20315. *Horse-sickness (mixed type). Died* 4/6/32.
Temperature Chart IX.



History: Horse 20315. An aged bay gelding, in good condition. Was placed on temperature 29/3/32 and was used in various mosquito transmission experiments (Messrs. Bedford and du Toit) and on 27/5/32 received intrajugularly 5 c.c. blood from Horse 20302 (*vide*). On the 3rd day p.i., the temperature reaction set in, rising to 105° within 24 hours, the horse succumbing on the 7th day p.i. from mixed type of horse-sickness. At the post-mortem examination was found cyanosis of the mucous membrane, transudation into the subcutis and loose connective tissue, marked oedema and hyperaemia of the lungs, subepicardial and subendocardial haemorrhages and degeneration of the myocard, stasis of the liver, strongylosis infection of the colon, and fatty degeneration of the kidneys.

TABLE 9.

Horse 20315.

Date		20/4/32.	4/5/32.	27/5/32.	1/6/32.	2/6/32.
Time		—	—	—	—	7.15 a.m.
Temperature Reactions		N	N	N	R	R
Hb. gm. %		16·29	11·67	10·60	10·27	10·81
Sugar mgm. %	L	80·65	80·50	91·74	109·90	96·15
	U	72·46	77·52	82·64	96·15	84·74
T.N. gm. %		3·116	2·710	2·402	2·430	2·437
N.P.N. mgm. %	L	25·00	23·08	23·08	21·43	18·60
	U	18·75	18·07	19·11	16·21	14·42
Coag. N gm. N %	L	3·091	2·687	2·379	2·409	2·418
	U	3·097	2·692	2·383	2·414	2·423
Urea mgm. N %	L	12·33 25·83	10·77 22·68	11·12 23·31	9·00 18·90	7·89 16·59
	U	11·50 24·15	10·24 21·42	10·72 22·47	8·75 18·27	7·73 16·17
Total Creatinine mgm. N %	L	2·06 5·54	1·91 5·14	2·27 6·16	1·78 4·80	1·91 5·14
	U	1·91 5·14	1·67 4·50	2·01 5·40	1·67 4·50	1·61 4·32
Uric acid mgm. N %	L	0·30 1·91	0·23 0·69	0·24 0·73	0·25 0·76	0·19 0·57
	U	— —	0·15 0·46	0·15 0·45	0·23 0·69	0·11 0·34
Amino acid mgm. N %	L	8·54	7·00	6·51	6·67	5·00
	U	5·38	4·38	4·38	3·89	2·80
R.N. mgm. N %	L	1·77	3·17	2·94	3·73	3·61
	U	—	1·63	1·85	1·67	2·18
Plasma		n.u.	n.u.	n.u.	n.u.	Slightly orange

Main Features of Analytical Data.

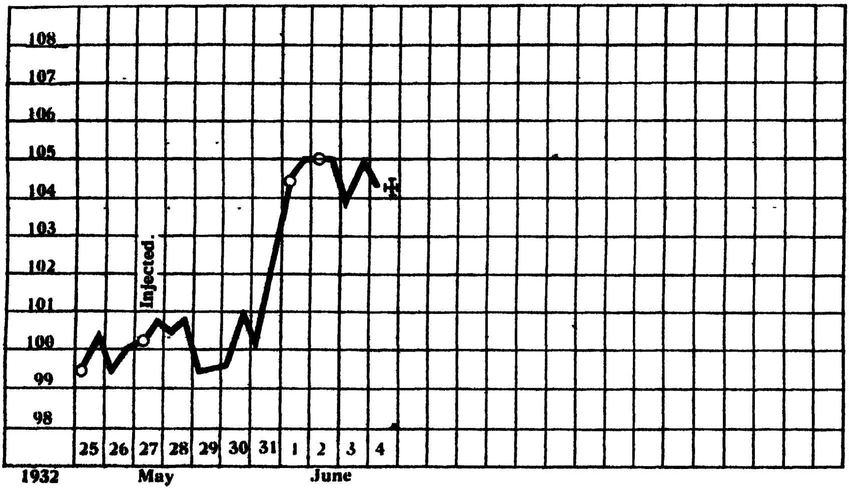
Sugar.—Shows a slight increase at beginning of temperature reaction.

N.P.N., *U.N.*, and *A.A.N.*—Show a slight decrease towards approach of death.

H.B., *T.C.N.*, *U.A.N.*, *R.N.*, and *T.N.*—No changes noted.

CASE X.

Horse 20280. *Horse-sickness* (*Dikkop*). *Died* 4/6/32.
Temperature Chart X.



History : Horse 20280. Chestnut gelding, six years old, in good condition and of a somewhat wild temperament. Placed on temperature on 24/11/31 and used in mosquito transmission experiments. The temperature remained normal throughout. On 27/5/31 the animal was injected subcutaneously with 1 c.c. blood from Horse 20302 (*vide*). On the 4th day p.i., the temperature reaction set in, the horse succumbing on the 9th day p.i. from Dikkop horse-sickness. At the post-mortem examination marked putrefactive changes were already found to be present, but the chief changes associated with horse-ickness could still be determined, such as gelatinous transudation, subserous petichiae, subendocardial haemorrhages and hydropericard.

Horse 20280.

TABLE 10.

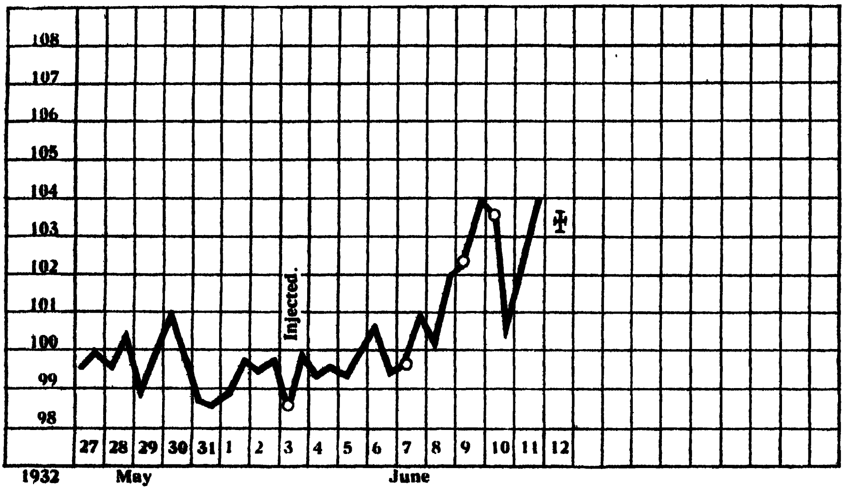
Date *Time*		30/11/31. —	4/12/31. —	4/3/32. —	9/3/32. —	11/3/32. —	16/3/32. —	20/4/32. —	25/5/52. —	27/5/32. —	1/6/32. —	2/6/32. 7.15 a.m.
Temperature Reactions		N	N	N	N	N	N	N	N	N	R	R
Hb. gm. %		13·31	13·25	15·42	16·87	15·19	15·19	15·21	18·20	16·87	14·95	—
Sugar mgm. %	L	92·60	84·74	90·91	82·64	106·40	100·00	97·07	80·00	90·09	—	117·64
	U	81·97	69·94	91·75	70·92	83·30	86·96	83·33	50·00	81·30	—	102·04
T.N. gm. %		2·964	2·850	2·989	3·248	3·115	3·325	3·144	3·479	3·507	3·186	—
N.P.N. mgm. %	L	26·08	22·25	23·55	25·60	23·10	23·10	25·00	22·81	21·90	20·71	20·57
	U	22·40	16·94	18·60	20·00	17·00	16·72	18·75	16·51	17·65	13·95	13·32
Coag. N mg. N %	L	2·938	2·828	2·965	3·222	3·092	3·302	3·119	3·456	3·485	3·165	—
	U	2·942	2·833	2·970	3·228	3·098	3·308	3·125	3·462	3·409	3·172	—
Urea mgm. N %	L	12·30	9·60	10·10	11·50	9·50	7·44	11·50	8·30	9·10	6·75	6·52
,, U %		25·83	20·16	21·21	24·15	19·95	15·54	24·15	17·43	19·11	14·28	13·65
mgm. N %	U	12·20	9·20	10·00	11·30	9·30	7·44	10·72	8·30	8·52	6·52	6·35
,, U %		25·62	19·32	21·00	23·73	19·63	15·54	22·47	17·43	17·85	13·65	13·44
Total Creatinine												
mgm. N %	L	2·48	2·48	2·11	2·30	2·23	2·42	2·13	2·23	2·66	2·11	2·27
,, TC %		6·70	6·70	5·68	6·20	6·00	6·54	5·76	6·00	7·20	5·67	6·16
mgm. N %	U	2·00	2·20	2·10	2·10	1·67	1·91	1·78	1·54	2·17	1·62	1·61
,, TC %		5·54	6·00	5·68	5·70	4·50	5·14	4·80	4·16	5·84	4·32	4·32
Uric acid mgm. N %	L	0·44	0·48	0·37	0·37	0·34	0·37	0·41	0·34	0·30	0·38	0·25
,, UA %		1·33	1·43	1·10	1·10	1·03	1·10	1·24	1·03	0·91	1·05	0·75
mgm. N %	U	0·21	0·21	0·30	0·25	0·16	0·23	0·22	0·16	0·15	0·23	0·13
,, UA %		0·64	0·64	0·89	0·75	0·48	0·69	0·67	0·49	0·46	0·69	0·40
Amino acid mgm. %	L	6·67	7·00	7·80	9·03	8·43	8·24	10·00	8·75	7·78	6·25	5·60
	U	5·71	4·38	4·20	4·40	4·40	4·52	4·83	3·89	4·67	4·00	2·50
R.N. mgm. N %	L	4·20	2·69	3·18	2·40	2·60	4·63	1·00	3·19	2·06	4·22	5·93
	U	2·30	1·00	2·00	1·95	1·47	2·62	0·20	2·62	2·14	1·59	2·74
Plasma		n.u.	n.u.	n.u.	n.u.	n.u.	n.u.	n.u.	n.u.	Orange	Orange	Slightly orange

Main Features of Analytical Data.

Sugar.—Shows an increase during the temperature reaction.
U.N., *U.A.N.*, and *A.A.N.*—A relatively slight drop is noted just prior to death.
R.N.—A slight increase is noted.
Hb., *N.P.N.*, *T.C.N.*, and *T.N.*—No changes noted.

Case XI.

Horse 20291. *Horse-sickness* (*Dunkop*). *Died* 12/6/32.
Temperature Chart XI.



History: Horse 20291. A black gelding, nondescript breed. Aged and in poor condition. The horse was placed on temperature 11/12/31. This horse was then used for mosquito transmission experiments (Messrs. Bedford and du Toit) and with the exception of a slight temperature reaction from the 15th to the 24th of February, 1932, of an undetermined nature, showed a very even normal temperature course. On 3/6/32, 1 c.c. blood was injected from Horse 20302 (Kaalplaas strain). The symptoms shown were those of a typical Dunkop horse-sickness reaction, the temperature reaction beginning on the 4th day p.i. On 10/6/32, 0·002 gm. Aciron per Kg. were injected for experimental purposes (Mr. Parkin) which was followed within 12 hours by a drop of temperature from 104°–100·4°, but during the next 24 hours the temperature rose again to 104°, the animal dying on the 12th of June (i.e., the 9th day p.i). On post-mortem examination was found to be present cyanosis of the mucous membranes, transudation into the subcutis, marked hydrothorax, hydropericard, severe oedema and hyperaemia of the lungs, subendocardial haemorrhages, degeneration of the myocard, fatty degeneration and hyperaemia of the liver and slight post-mortem changes of the kidneys.

TABLE 11.

Horse 20291.

Date	20/4/32.	26/4/32.	3/6/32.	7/6/32.	9/6/32.	10/6/32.
Time	—	—	7.15 a.m.	—	—	—
Temp. R	N	N	N	P.I.N.	R	R
Hb. gm. %	14·72	17·18	14·08	11·39	11·39	12·59
Sugar mgm. % L	82·64	108·70	70·42	77·52	102·10	—
U	70·42	100·00	60·61	66·67	90·91	—
T.N. gm. %	2·654	3·116	2·906	2·584	2·584	2·682
N.P.N. mgm. % L	28·85	28·56	21·43	24·59	22·75	20·98
U	21·75	20·83	15·79	19·14	18·07	15·28
Coag. N gm. % L	2·625	3·087	2·885	2·559	2·561	2·661
U	2·632	3·095	2·890	2·565	2·566	2·667
Urea						
mgm. N % L	13·18	11·50	6·87	11·20	10·24	7·77
„ U %	27·72	24·15	14·49	23·52	21·42	16·38
mgm. N % U	12·33	10·77	7·06	10·72	9·52	7·62
„ U %	25·83	22·68	14·91	22·47	19·95	15·96
T.C.						
mgm. N % L	1·98	1·78	2·27	2·42	1·98	—
„ TC %	5·32	4·80	6·16	6·54	5·32	—
„ N % U	1·78	1·49	1·78	2·01	1·67	—
„ TC	4·80	4·00	4·80	5·40	4·50	—
Uric acid						
mgm. N % L	0·62	0·45	0·38	0·37	0·38	—
„ UA %	1·86	1·34	1·14	1·12	1·14	—
mgm. N % U	0·37	0·27	0·22	0·29	0·27	—
„ UA	1·10	0·80	0·65	0·87	0·80	—
Amino acid mgm N % L	9·33	8·75	5·74	5·83	6·01	5·71
U	5·49	5·18	3·50	3·50	3·16	3·18
R.N. mgm. N % L	3·74	6·08	6·17	4·77	4·14	7·50*
U	1·78	3·12	3·23	2·62	3·45	4·48*
Plasma	—	—	—	—	—	—

* Includes "Total Creatinine Nitrogen" and "Uric Acid Nitrogen."

Main Features of Analytical Data.

Hb.—A slight decrease is observed.

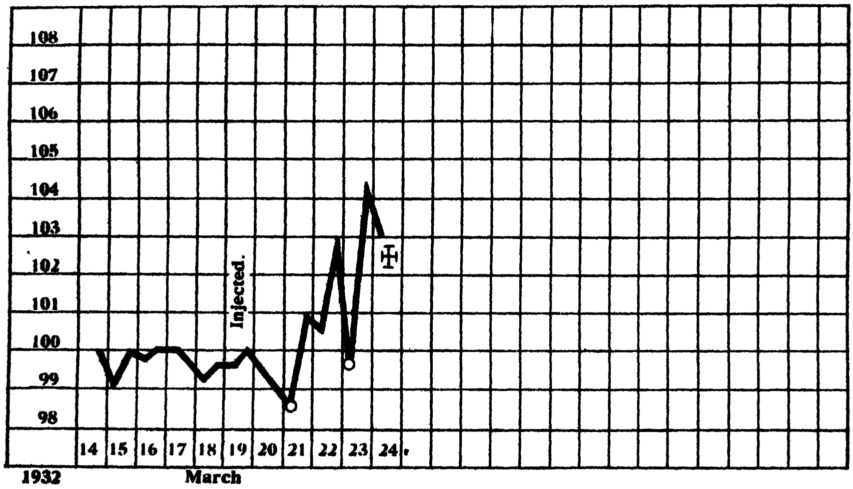
Sugar.—A slight increase is observed.

R.N.—In this particular case the R.N. throughout the period of examination is on the high side for both filtrates. The horse was several times clinically examined, but no explanation can be offered for the relatively high R.N. found.

N.P.N., U.N., T.C.N., U.A.N., A.A.N., **and *T.N.***—No changes noted.

CASE XII.

Horse 20270. *Horse-sickness* (*mixed type*). *Died* 24/3/32.
Temperature Chart XII.



History: Horse 20270. An aged bay gelding, in fair condition. Placed on temperature 23/11/31 and used for mosquito transmission experiments (Messrs. Bedford and du Toit). The temperature remained normal except for a slight reaction from 20/2/32–26/2/32, probably as a result of an injection with crushed mosquitos. At the site of the injection an abscess several cm. in diameter developed. On 9/3/32 this horse received intrajugularly 5 c.c. blood from Horse 20302 (*vide*) the temperature reaction setting in on the 2nd day p.i., the animal succumbing on the 5th day p.i. from a mixed horse-sickness reaction. At the post-mortem examination the diagnosis was confirmed by the presence of a marked transudation into the subcutis and loose connective tissues, a marked hydropericard, oedema of the lungs, subendocardial haemorrhages and fatty degeneration of the liver.

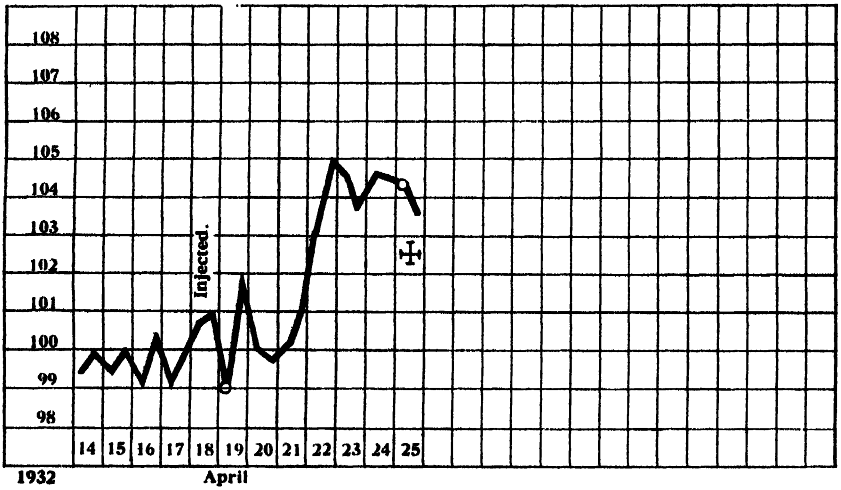
			TABLE 12. *Horse* 20270.		TABLE 13. *Horse* 20307.		TABLE 14. *Horse* 20319.		
Date			21/3/32.	23/3/32.	19/4/32.	25/4/32.	21/4/32.	22/4/32.	25/4/32.
Time			—	—	—	—	—	—	—
Temperature Reaction			PI.N.	R	P.I.N.	R	N	P.I.N.	R
Hb. gm. %			19·72	12·13	16·54	17·84	16·87	19·33	16·29
Sugar mgm. %		L	82·00	86·96	—	80·65	67·57	94·36	116·28
		U	74·63	82·64	—	69·44	—	79·36	107·50
T.N. gm. %			2·962	2·458	3·312	3·060	3·326	3·578	3·051
N.P.N. mgm. %		L	23·08	24·03	27·27	25·00	31·66	30·00	26·84
		U	17·97	17·57	16·66	16·77	23·08	24·26	19·61
Coag. N. gm. N %		L	2·939	2·434	3·285	3·035	3·294	3·548	3·024
		U	2·944	2·440	3·295	3·047	3·308	3·554	3·031
Urea	mgm. N %	L	10·24	9·25	8·13	7·00	14·03	15·00	10·70
	,, U %		21·42	19·53	17·00	14·70	29·40	31·50	22·47
	mgm. N %	U	10·11	9·25	7·33	6·66	13·29	14·38	9·81
	,, U %		21·21	19·53	15·33	14·07	27·93	30·24	20·58
Total Creatinine	mgm. N %	L	2·23	2·23	1·97	1·78	1·98	1·91	1·78
	,, TC %		6·00	6·00	5·32	4·80	5·32	5·14	4·80
	mgm. N %	U	1·84	1·78	1·41	1·52	1·30	1·41	1·30
	,, TC %		4·96	4·80	3·78	4·10	3·60	3·78	3·60
Uric acid	mgm. N %	L	0·29	0·27	0·35	0·37	1·89	1·04	0·78
	,, UA %		0·88	0·87	1·05	1·10	5·66	3·18	2·34
	mgm. N %	U	0·30	0·48	0·22	0·18	0·18	0·40	0·30
	,, UA %		0·89	0·16	0·67	0·54	0·53	1·19	0·89
Amino acid mgm. N %		L	7·00	5·66	11·66	10·00	8·75	10·00	8·43
		U	4·52	3·50	3·68	3·89	4·80	4·67	4·12
R.N. mgm. N %		L	3·32	6·65	5·16	5·85	5·05	2·06	5·08
		U	1·20	2·88	4·02	4·52	3·51	3·40	4·08
Plasma			—	—	n.u.	Orange yellow	n.u.	Orange	Orange

Main Features of Analytical Data.

In the case of these three horses no "normal" values were determined before the injection of virus and to establish what is "abnormal", is therefore, somewhat difficult, particularly since the normal variations are relatively large. With the exception of a tendency towards an increase in sugar in Case XIV and figures for R.N. rather on the high side of normal, no specific alterations can be noted.

CASE XIII.

Horse 20307. *Horse-sickness* (*Dikkop*). *Died* 25/4/32.
Temperature Chart XIII.



History: Horse 20307. An aged grey gelding, in poor condition. Was placed on temperature on 14/3/32 and received an intrajugular injection of 5 c.c. blood from Horse 20265 (O.virus 191st generation) on the 18th April, 1932. The temperature reaction set in on the 4th day p.i., reaching 105° within 24 hours, the animal dying on the 7th day p.i. It showed typical symptoms of Dikkop horse-sickness.

The post-mortem revealed that post-mortem changes were fairly well advanced but the gross alterations associated with Dikkop horse-sickness were present.

CASE XIV.

Horse 20319.

No Temperature Chart.

A bay gelding, aged, in fair condition. Was injected for virus purposes on 22/4/32, but was too wild at the start to submit to temperature taking. On the 5th day the temperature was 105·6°, The horse was then shot. At the post-mortem there was found to be present, hydropericard, fibrinous pleuritis, chronic fibrinous peritonitis and verminosis.

(*d*) NORMAL RANGE OF VALUES FOR Hb., SUGAR. T.N., N.P.N., U.N., T.C.N., U.A.N., A.A.N., AND REST N.

For the purpose of compilation of the normal values, only data obtained prior to the infection with virus has been considered here. During the determinations every endeavour was made to work as accurately as possible and to check the results by means of duplicate analyses. Where the duplicates were at variance with each other, a triplicate aliquot was analysed in the majority of cases. The data recorded throughout this article are, therefore, as accurate as the limitations of the methods permitted.

Unfortunately, the number of "normal" figures I am able to submit is relatively small (40-50 only), and have been collected from animals of various breeds and ages and taken at different times of the year. My only reason for publishing them here at this stage is that they have been obtained from the same animals which were subsequently infected, which received the same diet, lived in the same environment and because a basis for evaluation is so essential for the interpretation of the pathological data collected during these researches. A few figures are also included from animals not tabulated here, but which were examined several times in succession but later were found to be immune.

Haemoglobin.—The maximum variations encountered ranged from 10·64–20·18 gm. Hb. per cent. The range of variation is more clearly demonstrated hereunder by tabulating the number of times the "Hb. gm. per cent." fell into the specified groups.

"*Hb. gm.* %."	*Occurrence.*	"*Hb. gm.* %."	*Occurrence.*
9-10	0	15–16	11
10-11	1	16–17	8
11–12	2	17–18	4
12–13	0	18–19	3
13-14	4	19-20	1
14-15	7	20-21	1

The average range is, therefore, from about 13-19 gm. Hb. per cent. with a narrower limit of 14–17 gm. Hb. per cent.

The variation in the normal Hb. content of individual animals over a given period is, as a rule, more restricted, e.g., variations are in Case IV: 13·31–17·51 gm. Hb. per cent.; in Case VI: 14·95–17·18 gm. Hb. per cent.; in Case X: 13·25-19·15 gm. Hb. per cent.; and in Case VII: 14·72–16·87 gm. Hb. per cent.

Neser (1923) drew attention to similar variations in his researches on the "Percentage volume or count of red cells" from one and the same horse over short periods, and on the basis of numerous counts in various types of horses, after a discussion of the factors involved, comes to the conclusion that:—

"(1) Moderate periods of starvation, thirst or exercise, do not influence the percentage volume of the red corpuscles of jugular blood to any appreciable extent for any length of time; (2) Food or water, after moderate periods of stravation or thirst, probably causes a slight and very temporary increase or decrease in the percentage volume in the jugular vein; (3) The mechanical state of the circulation is a very important factor influencing the percentage volume or count of the red cells in any part; a slow peripheral circulation results in concentration there and dilution in the jugular vein; (4) Variations occur in the percentage volume or count of the red cells of the blood of the same animal on different dates and are due to factors which so far have not been controlled."

Neser referred here more particularly to variations encountered in the same animal at the same time in different parts of the circulation (venous and capillary), but his results obviously also apply to differences found in the same animal from day to day (in the absence of pathological conditions). When bleeding animals at short intervals, even at the same time on each day, we cannot for a moment assume that the physiological state of the circulation is exactly the same as at the previous bleeding, and that the Hb. figure obtained is, therefore, an " absolute " index to the Hb. content and that any variations would represent an absolute increase or decrease in the total amount of Hb. in the circulation. An absolute decrease or increase under normal conditions as a result of physiological adaptation to environment (e.g., altitude) increased or decreased work, etc., would be relatively gradual. Even under normal conditions, however, such absolute changes are possible, if one remembers that erythrocyte destruction and regeneration is a continuous process and unless these two processes are extremely delicately adjusted to each other at all times, variations in the Hb. content occur, even if only to a slight extent.

Sugar.—For " laked " filtrates the maximum variation was found to be from 57·47–119·10 mgm. per cent. with 80 per cent. of the data falling within the 70–100 mgm. per cent. range: for " unlaked " filtrates the corresponding ranges are from 54·30–100 mgm. per cent. and 65–90 mgm. per cent. (70 per cent.). The following table reflects the distribution more clearly:—

" *Laked* " *Sugar mgm.* %.	*Occurrence.*	" *Unlaked* " *Sugar mgm.* %.	*Occurrence.*
55– 60	1	50– 55	2
60– 65	1	55– 60	6
65– 70	3	60– 65	1
70– 75	5	65– 70	5
75– 80	1	70– 75	6
80– 85	11	75– 80	2
85– 90	3	80– 85	10
90– 95	6	85– 90	3
95–100	4	90– 95	1
100–105	2	95–100	2
105–110	2		
110–120	2		

Although wide variations may be experienced in one and the same animal, it is the exception, the variations being rather individual in this sense, that in some animals the " mean " level is higher, in others lower.

There is a considerable difference between the blood sugar content of " laked " and " unlaked " filtrate, as obtained by this method, the differences being from 3·3–37·5 per cent. with an average of 16 per cent., the " laked " filtrate being always the higher. The average obtained by the addition of *all* the normal figures and dividing by the number of analyses is 89·80 mgm. per cent. and 74·80 mgm. per cent. for " laked " and " unlaked " filtrate, respectively.

If, as is generally assumed, the distribution of blood sugar between plasma and corpuscles is approximately equal, the only conclusion to be arrived at is that with the destruction of the cellular elements other reducing substances are liberated and these affecting the total " sugar " percentage.

Non-Protein Nitrogen.—In the case of N.P.N., the maximum variation is from 18·65 mgm. N per cent.–31·66 mgm. N per cent. for "laked" and from 13·63 mgm. N per cent.–23·08 mgm. N per cent. for "unlaked" filtrate. The following table indicates the distribution:—

Laked.

mgm. N %.	*Occurrence*	*mgm. N %.*	*Occurrence*
18–19	2	25–26	5
19–20	4	26–27	3
20–21	3	27–28	3
21–22	4	28–29	2
22–23	4	29–30	0
23–24	7	30–31	0
24–25	3	31–32	1

Unlaked.

mgm. N %.	*Occurrence*	*mgm. N %.*	*Occurrence*
13–14	2	20–21	3
14–15	4	21–22	2
15–16	5	22–23	1
16–17	5	23–24	1
17–18	5		
18–19	10		
19–20	1		

From the above it can be seen that the majority of data falls into the groups 18–28 mgm. N per cent and 14–22 mgm. N Per cent. for "laked" and "unlaked" filtrates, respectively. The average is 18·9 mgm. N per cent. and 17·3 mgm. N per cent. respectively. The percentages variation between "laked" and "unlaked" is from 14·2–34·6 per cent. with an average of 25·1 per cent. For the N.P.N. an individual variation is also noticeable, i.e., animals are encountered who have either a relatively high or a relatively low normal N.P.N. level, e.g., c.f., Case IV with 18·64–24·04 mgm N per cent., Case V with 24·4–27·8 mgm. N per cent., Case VI with 19·4–22·9 mgm N per cent., and Case X with 22–26 mgm. N per cent.

Urea.—The normal urea nitrogen content yields maximum variations of from 4·7–14 mgm. N per cent (9·9–29·4 mgm. Urea per cent.), and from 4·4–13·3 mgm. N per cent. (9·3–27·9 mgm. Urea percent.), respectively, for "laked" and "unlaked" filtrates. To indicate the distribution more accurately, the following table is attached, but owing to the small variations between the urea nitrogen content in the two filtrates, all the analytical data has been incorporated in the one column.

mgm. U.N. %.	*Occurrence*	*mgm. U.N. %.*	*Occurrence*
4–5	2	10–11	14
5–6	4	11–12	10
6–7	15	12–13	6
7–8	8	13–14	3
8–9	8	14–15	1
9–10	13		

It will be noted that the majority of analytical figures lie between 6–13 mgm. N per cent. (12·6–27·3 mgm. Urea per cent.) with the maximum at ± 10 mgm. N per cent. (23·1 mgm. Urea per cent.). If the average of all the “ laked ” and “ unlaked ” is calculated, the figures 9·3 mgm N per cent. and 8·8 mgm N per cent., respectively, are obtained. The variations encountered are also more or less individual, i.e., each animal within the prescribed limits has its own mean “ U.N.” content, which is either relatively low, c.f., Case IV with 6·24–9·87 mgm N per cent.; Case VI with 4·7–7·3 mgm. N per cent.; and Case VIII with 9·25–11·58 mgm. N per cent.

Totale Creatinine Nitrogen.—The normal range is from 1·52–2·80 mgm. T.C.N. per cent. (4·1–7·6 mgm T.C. per cent.) and 1–2·23 mgm. T.C.N. per cent. (3·0–6 mgm. T.C. per cent.) with an average of 2·1 mgm T.C.N. per cent. and 1·8 mgm. T.C.N. per cent. for “ laked ” and “ unlaked ” filtrates, respectively. The following table gives a more accurate conception of the range of distribution :—

“ Laked.”		*“ Unlaked.”*	
mgm. T.C.N. %.	*Occurrence*	*mgm. T.C.N. %.*	*Occurrence*
1·50–2·00	12	1·00–1·50	9
2·00–2·50	27	1·50–2·00	17
2·50–3·00	2	2·00–2·50	14

From this it is evident that the mean T.C.N. concentration lies from 2·00–2·50 mgm. T.C.N. per cent. (5·4–6·8 mgm. T.C. per cent.). The percentage differences between the “ laked ” and “ unlaked ” concentrations vary from 0–35 per cent. with an average of 16 per cent.

Although variations exist the relatively most constant values of any of the nitrogenous fractions sought for were found in this group, e.g., in Case IV, the date is 2·42, 2·42, 2·42 and 2·01 and 2·23 mgm. for “ laked,” and 2·23, 2·23, 2·23, 2·23 and 2·10 mgm. “ unlaked.”

As was shown to be the case in the previously discussed constituents, each individual has its own particular level, e.g., Case VI: 2·06, 1·91, 1·67, 1·52, 1·91, 1·98 for “ laked,” and Case IV: 2·42, 2·42, 2·42, 2·01, 2·23 for “ laked,” respectively.

Uric Acid.—The normal variations encountered ranged from 0·23–0·82 mgm. U.A.N. per cent. (0·70–2·5 mgm. U.A. per cent.) and 0·13–0·53 mgm. U.A.N. per cent. (0·40–1·6 mgm. U.A. per cent.) in “ laked ” and “ unlaked ” filtrate respectively. The average figures are 0·41 mgm. T.C.N. per cent. and 0·25 mgm. T.C.N. per cent.

The following table indicates the number of times each particular concentration was met with :—

“ Laked.”		*“ Unlaked.”*	
U.A.N. mgm. %.	*Occurrence*	*U.A.N. mgm. %.*	*Occurrence*
0·20–0·30	12	0–0·10	0
0·30–0·40	8	0·10–0·20	15
0·40–0·50	12	0·20–0·30	12
0·50–0·60	3	0·30–0·40	6
0·60–0·70	2	0·40–0·50	3
0·70–0·80	2	0·50–0·55	1
0·80–0·82	1	0·50–0·60	0

From this the majority of figures are seen to range from 0·20–0·50 mgm. U.A.N. per cent. and from 0·10–0·40 mgm. U.A.N. per cent. The differences between the "laked" and "unlaked" range from 0 per cent. to 70 per cent., with an average of 36·5 mgm. per cent., the "laked" content being the higher. Each individual has its own particular level. This clearly emerges from Case VIII, where the average U.A.N. per cent. is 0·25 mgm. and Case X where it is 0·38 mgm. and Case VI where is is 0·48 mgm. N per cent. and correspondingly less in the "unlaked" filtrates.

Amino Acids.—This fraction ranges from 5·38–10·80 mgm. N per cent. and from 3·50–5·83 mgm. N per cent. with an average of 7·90 mgm. N per cent. for "laked" and 4·43 mgm. N per cent. for "unlaked." This fraction appears to be the only nitrogenous fraction differing from the others in so far as no "individual" level appears to exist.

Rest Nitrogen.—The R.N. (or "undetermined" nitrogen) here represents that portion of the N which remains after the U.N., U.A.N., T.C.N., and A.A.N. have been subtracted from the N.P.N. It is obtained by calculation and, therefore, its accuracy depends on the accuracy of each determined N fraction.

In the case of the horse the maximum variations range from 1–6·66 mgm. N per cent. and from 0·20–4·44 mgm. N per cent. in "laked" and "unlaked" filtrates, respectively. The percentage differences range from ± 10 per cent. to ± 60 per cent., with an average of 43·3 per cent. Between "laked" and "unlaked" respectively.

The "average" R.N., taking all the normal data into consideration, is 3·70 mgm. N per cent. and 2·11 mgm. N percent., respectively, the "laked" being in the vast majority of cases higher.

Total Nitrogen.—The T.N. showed variations from 2·7 gm. N per cent.—3·6 gm. N per cent. with an average of—3 gm. N per cent., depending partly on the Hb. content. There is, therefore, also an individual level for T.N. which, under relatively constant conditions, remains fairly constant for each particular animal.

(*e*) General Summary of Range of Values during Horse-sickness.

This summary of the composition of blood gives the essential variations found in fourteen cases of horse-sickness of which six cases were of the Dikkop form, three of the Dunkop form, three of the mixed form, and one animal was shot before pathognomic symptoms could be noted, i.e., out of fourteen cases only one recoverd. It is, therefore, evident that on the score of virulency, nothing remained to be desired.

Analyses were performed frequently: during the reaction every 48 hours, frequently every 24 hours, till death supervened. Considering the constituents one by one, the following main features were observed during the course of the reactions.

Haemoglobin.—This showed no characteristic differences from normal. In some cases (Ix, X, XIII, XIV) the Hb. content remained unchanged throughout; in others a slight decrease could be seen (III, XI, XII) in others, on the other hand, a slight increase (IV and VI). Some few cases showed a slight drop followed by an increase (I and VIII); others again the reverse (II and VI). The highest Hb. content appeared in two horses bled one and four hours, respectively, before death. (23 and 25 gm. Hb. per cent.). In *all* the other animals the variations *were within the normal range* and may, therefore, have been coincidental, although I believe that they are associated in some way with the horse-sickness reaction.

This disease is not in the true sense a blood disease such as one would regard anaplasmosis or piroplasmosis, and severe anaemias, therefore, do not occur unless, of course, complicated with infectious anaemia or biliary fever (*P. caballi* and *N. equi* infection) as a result of breakdowns of immunity. There would, however, appear to be a slight destruction of erythrocytes in the majority of cases as a change in the colour of the serum to an orange-yellow—indicative of icterus—is quite common. Furthermore, other factors would tend to upset the Hb. level such as e.g., the water retention so frequently found in fevers. This would tend relatively to decrease the Hb. content. The tremendous transudation and exudation into the lungs, pleural cavities and subcutis would lead to a concentration of the blood, even if only temporary, and thus to an increase of the Hb. index.

Sugar.—In eight cases (I, II, III, IV, V, X, XI, XIV) an increase in the blood sugar level took place; the maximum figure obtained being 161·3 mgm. per cent. (Case V) bled an hour before death. In the remaining cases the increases are only slightly above the upper level of normal, or even below it. The increase in such cases was only relative in comparison with the normal or preinfection content. In the remaining six cases no changes were detectable (VI, VII, VIII, XI, XII, XIII).

Total Nitrogen.—No specific alterations occur, minor increases and decreases being associated with the corresponding changes in the Hb. content.

Urea Nitrogen.—In most cases there is a tendency towards a slight decrease.

Non-Protein Nitrogen.—Shows no distinctive alterations.

Uric Acid Nitrogen.—Shows no distinctive alterations.

Amino Acid Nitrogen.—Shows no distinctive alterations.

Total Creatinine Nitrogen.—Shows no distinctive alterations.

Rest Nitrogen.—With the exception of Case VII where it is increased, no definite variations occur. Case V forms an exception in so far as the above statements are concerned. All the constituents on the last day (four hours before death) were increased considerably. This would appear to be due to inspissation of the blood and not due to specific changes associated with horse-sickness, since all the constituents are practically equally affected.

(*f*) CONCLUSION.

The changes in the composition of the blood encountered in horse-sickness are practically nil, and considering the severe symptoms and the striking post-mortem findings, the negative results are surprising. It is true that in most cases the disease ran a very acute course, and it may be urged that, therefore, the time period for a difference in composition to develop was too short. This may partially explain the virtually negative findings, but it should be pointed out that in the one case of recovery no more marked alterations occurred than in the others. One case is obviously insufficient to permit of any definite conclusions being drawn, and it is the intention to undertake analyses in " recovery cases " as apportunities occur. Very scant data exists of chemico-pathological blood examination in horses during other diseases, but conceivably an explanation for the relative stability of the composition of the blood finds a physiological explanation in the light of the peculiar development of the present day horse. Amongst all our domestic animals it is the fastest and most active, capable of heavy, long continued work, or spurts of intensive activity (race horses). For this a highly developed efficient circulation is a *sine qua non* and the maintenance of an effective mechanism for maintining the normal composition of the blood and the efficient removal of waste products is essential. Further chemical researches into the composition of the blood of the horse during health and disease are necessary before any explanation advanced becomes of more than argumentative interest.

Attention was drawn to the existence of " individual " levels for most of the normal nitrogenous fractions such as N.P.N., U.N., T.C.N., etc. These variations may be associated with such factors as condition, " fitness," stamina, temperament, age, sex, normal functional capacity of the kidneys, basic tissue, metabolism, etc., or undiagnosed chronic conditions, but without a greater mass of normal data collected for the special purpose of determining the causes of these variations, no explanation based on experimental evidence is possible.

This line of investigation would in my opinion, be of interest, enabling one possibly even to pick out a potential winner—or loser!—out of a bunch of race horses or a good milker-to-be out of a herd of yearling heifers, on the basis of accurate chemical blood examinations alone!

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JORDEN, T. J., AND GRAF, H., II. A contribution to the determination of urea in animal blood filtrates (" laked " and " unlaked ").

GRAF, H., III. Comparative studies on " laked " and " unlaked " blood filtrates of sheep in health and during Heartwater (*Rickettsia ruminantium* infection) and Bluetongue (Catarrhal fever).

GRAF, H., IV. This article.

GRAF, H., V. Comparative studies on " laked " and " unlaked " blood filtrates of cattle in health and during Anaplasmosis (*A. marginale*) and Piroplasmosis (*P. bigeminum*).

HAMERSMA, P. J., VI. A serial study (over a 12 month period) of some organic constituents in " laked " and " unlaked " blood filtrates of healthy sheep of various ages. (To appear in next number of this Journal.)

Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 1, Number 1, 1933.

Chemical Blood Studies.*

V. Comparative Studies on "Laked" and "Unlaked" Blood Filtrates of Bovines in Health and during Anaplasmosis (*A. marginale* infection) and Piroplasmosis (*P. bigeminum* infection).

By H. GRAF, B.Sc., D.V.Sc., Veterinary Research Officer, Department of Chemical Pathology, Onderstepoort.

CONTENTS.

I. INTRODUCTORY NOTE.

II. ANAPLASMOSIS OF CATTLE.
  (*a*) Symptomatology and Pathological Anatomy.
  (*b*) Methods of Analyses and Technique.
  (*c*) Experimental Data. (Temperature charts, tables of data and discussion.)
  (*d*) Normal Range of Values for Haemoglobin, Sugar, Total Nitrogen, Non-Protein Nitrogen, Urea, Total Creatinine, Uric Acid, Amino-Acid, and Rest Nitrogen.
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III. PIROPLASMOSIS OF CATTLE.
  (*a*) Experimental Data. (Temperature chart and table of data.)
  (*b*) Summary of Data.

IV. ACKNOWLEDGEMENTS.

V. REFERENCES.

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* Chemical Blood Studies I, III–V accepted as Thesis for the D.V.Sc. degree by the University of Pretoria, December, 1932. For the other titles of the series see under "References."

I.—INTRODUCTORY NOTE.

The present paper is one of a series of publications on researches into the composition of the blood of domestic animals, both in health and during the course of various infections, (stock diseases), occurring in South Africa. It embodies the results of the experimental work performed in connection with anaplasmosis and piroplasmosis. The aims, plan of research, methods of analyses used and general technique, arrangement of data, etc., have been fully set out in the first paper [Chemical Blood Studies I., page 269, of this Journal and in order to avoid needless repetition this aspect of the work has been omitted here.

II.—ANAPLASMOSIS OF CATTLE.

(*a*) SYMPTOMATOLOGY AND PATHOLOGICAL ANATOMY.

The disease investigated is that produced in susceptible animals by an infection with *Anaplasma marginale.*

The condition is briefly described here from a symptomalogical and pathological-anatomical point of view for the purposes of a clearer understanding of the correlation between the chemical blood changes and the disease complex. A bibliography, listing some of the more important publications on anaplasmosis, will be found at the end of this article. No references could be found in the available literature to any chemical researches into this condition.

Anaplasmosis has been defined (Theiler, 1910) as a disease of cattle, caused by a protozoön, *Anaplasma marginale*, which invades and destroys the red blood corpuscles causing primarily an acute oligocythaemia accompanied by high fever and secondly, a degeneration of all parenchymatous organs. Recovery from the disease gives resistence to subsequent infections. The immune animal acts as a reservoir for the virus and the blue tick (*Boophilus decoloratus*) acts as host or transmitter of the parasite." Subsequently various other ticks have been found capable of transmitting the disease.

The disease (Knuth and du Toit, 1921) can also be transmitted artificially through the subcutaneous, intravenous and intramuscular injection of infected blood into susceptible bovines.

The incubation period varies considerably, e.g. from 16–17 days by subinoculation, and 60–80 days with tick transmission. A fever reaction is usually the first symptom, followed by loss of appetite, loss of condition, anaemia, icterus, salivation and generally constipation, rarely diarrhoea. In severe cases the patient goes down. The urine is icteric, but very rarely blood stained. There is dyspnoea, an increased heart rate, and frequently muscular tremblings shortly before death. The severity of the symptoms vary considerably. They may be so mild as to virtually escape detection except for a slight fever reaction, or the patient may show all or most of the above symptoms, especially imported cattle. Calves show as a general rule only relatively mild symptoms.

Pathological-anatomically the following changes are usually noted: generalised icterus, gelatinous transudation into the tissues of the neck, sternum and abdomen; severe anaemia, and oligacythaemia, the blood being thin, watery and staining badly: subepicardial and occasionally subendocardial haemorrhages; tumor hepatis and yellow coloration due to bile stasis and fatty degeneration; enlarged gall bladder containing a viscid thick dark green bile; tumor splenis and subcapsular haemorrhages and impacted omasum.

Morphologically the blood, in addition to the presence of parasites, reveals marked anaemic changes, such as anisocytosis, polychromasia, basophilla, uncleated red cells (Jolly bodies) and a decreased erythrocyte count. In spite of severe erythrocyte destruction, haemoglobinuria very rarely occurs. The severity of the blood changes may vary from a slight anisocytosis in mild cases, to the above described changes in severe reactions.

(*b*) Methods for Analysis and Technique.

The same methods and technique as previously described in paper I of this series (p. 000), was adhered to. Bovine blood tends to disintegrate more rapidly during the preparation of " unlaked " filtrate, and as soon as sufficient filtrate has been obtained, the filter paper should be removed. This is particularly the case with pathological bloods.

(*c*) Experimental Data in Anaplasmosis.

The analytical figures submitted here were obtained from 9 cases of anaplasmosis, of which 3 proved fatal, 4 could be regarded as severe, and 2 as very mild. The transmission was always by means of subcutaneous inoculations of 5-10 c.c. infected blood, the animals being placed on temperature, blood smears examined frequently, and analyses made throughout the course of the disease. In all cases a " pure " strain of *A. marginale* was utilised, i.e. uncomplicated by the presence of the redwater parasite (*P. bigeminum*). In some cases chemotherapeutic treatment by means of subcutaneous or intravenous injections of various drugs was given in an endeavour to find an effective anaplasmocidal agent. This aspect of the problem was under the charge of Mr. Parkin of this Division, and in view of his intention to shortly publish his results on a large number of drugs tested out, I feel constrained to omit here publication of his data. I have, however, with his sanction indicated on the temperature charts the periods at which such injections were made. In a few cases a definite effect of the drug as evinced by the sudden drop in the temperature, in others no such visible effect is noticeable. Four patients were, however, untreated, and the analytical data obtained from them therefore represents the conditions as they are found in natural cases.

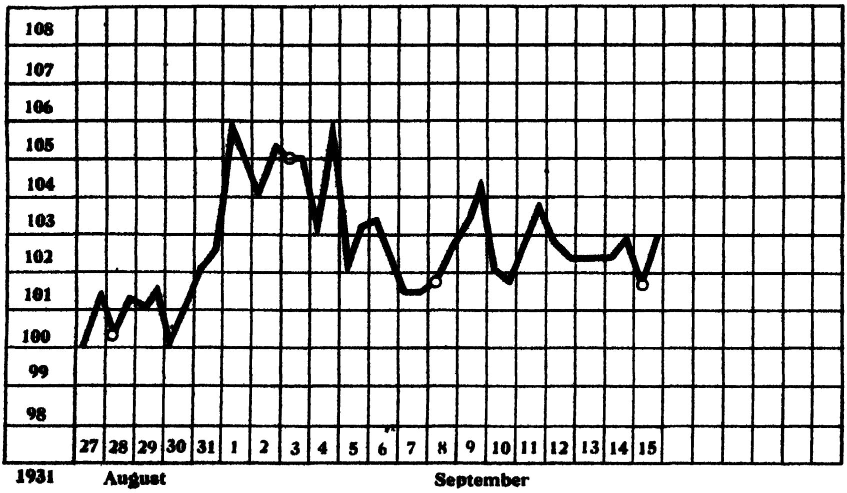
In the arrangement of the analytical data, the same sequence as previously used has been followed, i.e. the temperature record, a short history of the animal, symptoms shown, etc., the analytical data, followed by a brief discussion of the main abnormalities noted in the blood in each particular case. A general summary and a discussion of the findings concludes this section.

From the morphological aspect of normal bovine blood under South African conditions, I would refer to a paper by Canham (1930), in which the erythrocyte count, differential count and the influence of various factors such as exercise, pregnancy, altitude, lactation, age, etc., is discussed.

CASE I.

B. 2889. *Anaplasmosis* (*Treated and Recovered*).

Temperature Chart I.



History.—B. 2889, a young ox (6 tooth), received here from the Government Cattle Ranch, Messina, 15/2/29, and immunised against blackquarter in May, 1929. Negative for tuberculosis (tuberculin test in June, 1931). Was drafted into the anaplasmosis experiment, 18/8/31, receiving on even date subcutaneously 5 c.c. blood from B. 3056 which at the time was reacting with an *A. marginale* infection. On 31/8/31 the first few anaplasms were noted coinciding with the beginning of the hyperthermic reaction. The parasites became frequent, and the usual blood changes associated with this infection developed. The animal did not feed well at the beginning and showed anorexia for about seven days during the main reaction; the faeces became reduced in amount and firm in consistency. Was treated, 3/9/31, chemotherapeutically. By 25/9/31, i.e. 37 days p.i., the parasites had virtually disappeared, but moderate blood changes still persisted. Made an uneventful recovery and was discharged in December, 1931.

TABLE 1.—*B.* 2889. *Anaplasmosis* (*Treated*) *Recovered.*

		18/8/31.	20/8/31.	28/8/31.	3/9/31.	8/9/31.	15/9/31.	17/9/31.	22/9/31.	7/10/31.	15/10/31.	22/10/31.	11/11/31.
Date *Time*		—	—	—	—	—	—	—	—	—	—	—	—
Temp. Reaction		N	P.I.N.	P.I.N.	R	R	R	N	N	N	N	N	N
Haemoglobin		16·87	17·51	17·51	12·78	6·44	5·07	5·61	7·25	10·60	12·94	13·31	15·42
Sugar mgm. %	L U	74·10 68·50	82·6 76·8	90·9 82·6	52·91 54·64	— —	50·00 40·80	94·90 70·00	60·20 41·60	53·19 51·00	47·62 33·33	67·50 60·00	43·67 37·45
Total N. gm. %		3·025	3·400	3·305	2·955	2·094	1·884	2·017	2·200	2·773	2·920	2·892	3·046
N.P.N. mgm. %	L U	19·60 13·50	21·55 16·13	20·00 13·80	20·00 16·21	21·70 —	19·11 15·31	22·90 17·34	19·86 14·92	10·98 13·35	17·44 12·53	18·43 12·53	18·13 12·50
Coag. N. gm. N %	L U	3·005 3·011	3·378 3·384	3·285 3·291	2·935 2·939	2·073 —	1·865 1·869	1·994 2·000	2·180 2·185	2·752 2·760	2·903 2·907	2·874 2·879	3·028 3·033
Urea mgm. N % „ U %	L	6·40 13·44	8·50 17·85	5·72 11·97	7·20 15·12	— —	— —	— —	— —	6·15 13·02	5·13 10·71	6·22 13·02	5·26 11·13
mgm. N % „ U %	U	6·30 13·23	8·50 17·85	5·43 11·34	5·70 11·97	— —	— —	— —	— —	5·80 12·18	5·48 11·55	5·82 12·18	4·44 9·24
Total Creatinine mgm. N % „ TC %	L	— —	— —	— —	— —	— —	2·89 7·80	2·66 7·20	2·08 5·60	2·85 7·72	2·23 6·00	2·66 7·20	2·96 8·00
mgm. N % „ TC %	U	— —	— —	— —	— —	— —	2·66 7·20	2·36 6·40	— —	2·23 6·00	1·67 4·50	2·35 6·36	2·66 7·20
Uric acid mgm. N % „ UA %	L	0·43 1·30	0·52 1·56	0·44 1·33	0·50 1·50	0·30 0·90	0·28 0·83	0·60 1·80	0·38 1·14	0·35 1·05	0·53 1·60	0·46 1·39	0·52 1·55
mgm. N % „ UA %	U	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —
Amino acid mgm. N %	L U	7·20 4·40	6·40 4·40	6·50 4·00	6·63 4·88	7·00 —	6·93 5·38	9·21 5·00	8·38 5·30	5·45 3·64	6·90 5·00	5·45 3·64	5·38 4·12
Rest Nitrogen mgm. N %	L U	4·57* 3·50‡	6·13* 3·23‡	7·34* 3·37‡	5·67* 5·63‡	— —	9·00† —	10·43† —	9·00† —	6·18§ 1·68§	2·65§ 0·38§	3·64§ 0·72§	4·00§ 0·28§
Plasma		n.u.	n.u.	n.u.	icteric	icteric	icteric	icteric	n.u.	n.u.	n.u.	n.u.	n.u.

* Includes "Total Creatinine Nitrogen."
† Includes "Urea Nitrogen."
‡ Includes "Total Creatinine Nitrogen" and "Uric Acid Nitrogen."
§ Includes "Uric Acid Nitrogen."

Main Features of Analytical Data.

Hb.—A very striking decrease in Hb. content is noted during the fever reaction, the Hb. dropping from about 17 gm. to 5 gm. per cent. The erythrocyte destruction is relatively rapid, regeneration relatively slow, the original Hb. level not being quite reached eight weeks later. Haemoglobinaemia was never noted, but bilirubinaemia was present during the period of acute blood destruction.

U.A.N.—Shows a decrease during the reaction and only reaches its former level 3–4 weeks after the return of the temperature to normal.

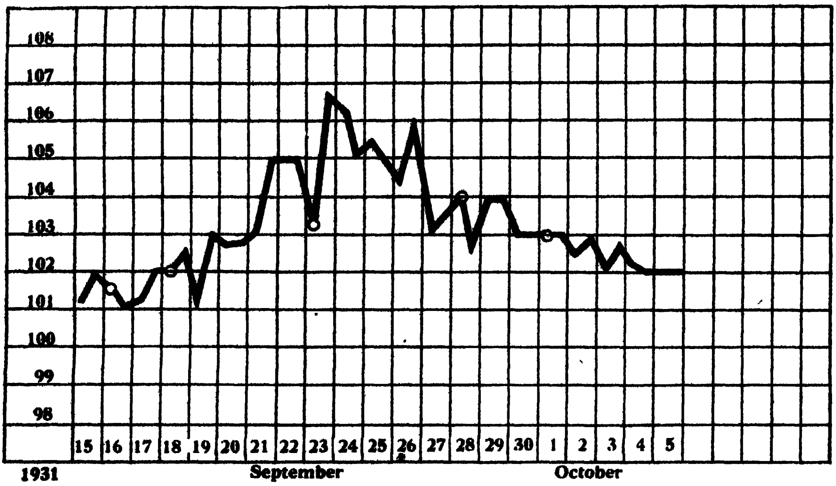
T.N.—Shows a drop coinciding with erythrocyte destruction from + 3 3–1 9 gm. per cent. within two weeks, followed by a gradual increase over the next eight weeks.

N.P.N., U.N., A.A.N., T.C.N., Sugar, and R.N.—Nothing unusual.

CASE II.

B. 3532. *Anaplasmosis* (*Treated and Recovered*).

Temperature Chart II.



History.—B. 3532. A four-year old ox, received from Government Ranching Station, Messina, on 7/6/29. In good condition. On 1/9/31 was injected with 10 c.c. blood subcutaneously from blesbok (*Damaliscus albifrons*) 32055, which was suffering from a pure *A. marginale* infection. On the 21st day p.i., the temperature reaction set in, the first parasites being noted on the 17th day p.i. and becoming numerous from the 21st to 32nd day p.i., thereafter gradually decreasing. Morphological blood changes became noticeable on the 25th day p.i. beginning with a slight anisocytosis and running the whole gamut of changes associated with an anaplasma infection, i.e. anisocytosis, polychromasia, basophilia, normoblasts, Jolly bodies, etc. By the 43rd day p.i. only a slight anisocytosis remained. During the hyperthermic reaction the usual symptoms such as lack of appetite, dullness, staring coat and slight icterus could be observed. On the 24th day p.i. an injection was given, for experimental chemotherapeutic purposes with apparently negative results, the temperature not being affected. Animal discharged 22/10/31.

TABLE 2.—*B.* 3532. *Anaplasmosis* (*Treated*) *Recovered.*

Date		1/9/31.	4/9/31.	9/9/31.	16/9/31.	18/9/31.	23/9/31.	28/9/31.	1/10/31.	7/10/31.	15/10/31.	22/10/31.
Time		—	—	—	—	—	—	—	—	—	—	—
Temperature R.		N	P.I.N.	P.I.N.	P.I.N.	P.I.N.	R	R	R	N	N	N
Haemoglobin gm. %		—	14·28	15·42	15 42	13·87	16 56	7·80	9·23	10·01	13·50	18·57
Sugar mgm. %	L	66·24	60·00	—	—	59·30	53·40	60·10	86·65	55·00	45·40	49·00
	U	48·00	50·00	—	—	56·20	38·00	—	70·00	45·00	31·50	42·00
Total N. gm. %		3·340	3 196	3·270	3·100	3·075	3 235	2 273	2·400	2·618	2·959	3·250
N.P.N. mgm. %	L	16·00	18·63	19 60	18·54	20·54	25·42	20·30	20·00	15·40	17·50	16·00
	U	11·70	15·55	15·73	15·00	16 83	21·00	17·14	14·23	12·80	13·97	12·50
Coag. N. mgm. N %	L	3·324	3·177	3 250	3·081	3·054	3·210	2·253	2·380	2·603	2·941	3·234
	U	3·328	3·180	3 254	3 085	3·058	3 214	2 256	2·386	2·605	2·945	3·237
Urea mgm. N %	L	3·92	—	—	5·20	7 10	10·50	10·56	6·90	4 40	4·83	4·88
„ U %		8·19	—	—	10 92	14·91	22·05	22·26	14·49	9 24	10·08	10·29
mgm. N %	U	3·50	—	—	4 88	7·00	10·30	10·17	6·35	4·19	4·79	4·88
„ U %		7·35	—	—	10·29	14·70	21·63	21·42	13·44	8·82	10·08	10·29
Total Creatinine mgm. N %	L	—	—	—	2 76	2 44	2·66	2·23	1·95	2·50	2·32	2·50
„ TC %		—	—	—	7 46	6·60	7·20	6·00	5·14	6 74	6·26	6·74
mgm. N %	U	—	—	—	2·23	1·82	2·35	2·23	1·75	2·10	2·01	2·23
„ TC %		—	—	—	6·00	5·00	6·36	6·00	4·70	5·68	5·40	6·00
Uric acid mgm. N %	L	0·53	0 47	0·50	0 52	0·53	0·60	0·31	0·27	0·31	0·41	0·51
„ UA %		1·60	1·40	1·50	1·57	1·60	1·80	0·94	0·80	0·93	1·23	1·64
mgm. N %	U	—	—	—	—	—	—	—	—	—	—	—
„ UA %		—	—	—	—	—	—	—	—	—	—	—
Amino acid mgm N %	L	5·26	5·09	4·84	5·57	6·33	5·80	4·76	6·17	6·17	7·00	4·90
	U	3·33	3 68	3 73	4·00	4·67	5·00	4·00	3·64	3·84	5·40	3·73
R.N. mgm. N %	L	—	—	—	4·55*	4·24*	6·48*	2·44*	4·71*	2·02*	2·94*	3·15*
	U	—	—	—	3 89*	3·89*	3·35*	1 74*	2·26*	2·67*	1·77*	1·66*
Plasma		n.u.	n.u.	n.u.	n.u.	n.u.	slightly icteric	slightly icteric	n.u.	n.u.	n.u.	n.u.

* Includes "Uric Acid Nitrogen."

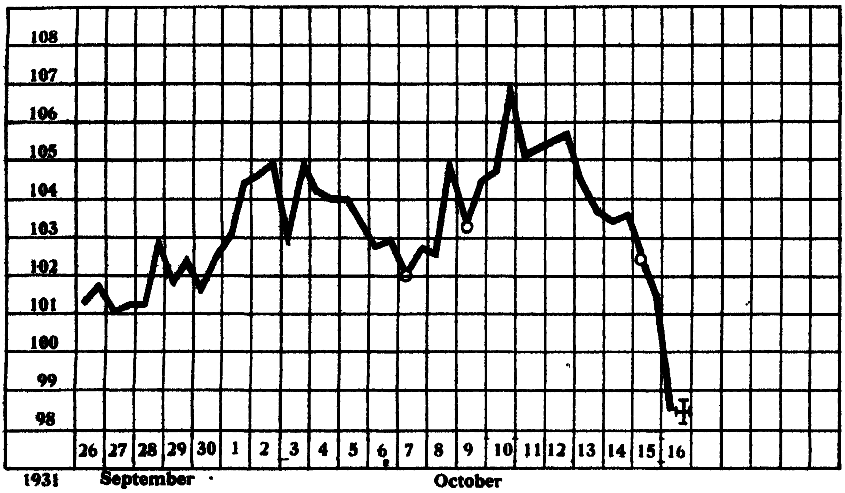
Main Features of Analytical Data.

Hb.—A decrease from 15–7·5 gm. % occurs within five days followed by a steady relatively rapid return to the normal level within the succeeding three weeks.
N.P.N.—An increase from 20–25 mgm. N % occurs at the height of the reaction, but the normal level is reached within a few days.
U.N.—Shows an increase from ± 5–11 mgm. N % coinciding with the increase in N.P.N.
U.A.N.—Exhibits a decrease from ± 0·5– ± 0·3 mgm. N % during the reaction, its previous level only being attained 3–4 weeks later.
T.N.—Shows a decrease from ± 3·3–2 2 gm. N % corresponding to the acme of Hb. destruction as reflected by the Hb. determination.
Sugar, T.C.N., A.A.N., R.N.—Nothing unusual.

CASE III.

B. 2888. *Anaplasmosis. Untreated—died* 16/10/31.

Temperature Chart III.



History: B. 2888. A black six-tooth ox in fair condition, bred on the Messina Government Cattle Ranch. Received here 15/2/29. Was inoculated against anthrax and blackquarter in March, 1931, and drafted into the anaplasmosis experiment 17/9/31, on which date it was injected subcutaneously with 5 c.c. blood of B. 2889 (*vide*). On the 14th day p.i. the temperature reaction set in. The animal went through a severe reaction, showing numerous anaplasms and severe morphological blood changes ; as well as marked clinical symptoms such as anorexia, dullness, icterus and severe anaemia. It died on the morning of the 30th day p.i. Post-mortem wás performed within 30 minutes of death and the following pathological-anatomical changes were noted : poor condition, anaemia, generalised icterus, subepicardial petechiae, a distended gall bladder containing a thick greenish yellow bile, fatty changes and bile stasis of the liver, tumor splenis, impaction of the omasum and catarrhal enteritis—findings which substantiated the clinical diagnosis.

TABLE 3.—*B.* 2888.

Date..........................		22/9/31.	7/10/31.	9/10/31.	15/10/31.
Time..........................		—	—	—	—
Temp. Reaction................		P.I.N.	R.	R.	R.
Haemoglobin gm. %............		12·94	12·01	13·31	4·33
Sugar mgm. %............	L	43·30	58·82	74·07	42·40
	U	33·40	42·37	64·10	36·40
Total N. gm. %................		2·913	2·857	2·548	1·730
N.P.N. mgm. %...........	L	23·17	22·73	19·73	53·38
	U	16·62	16·92	14·35	49·58
Coag. N. gm. N %.........	L	2·890	2·834	2·528	1·677
	U	2·894	2·837	2·534	1·680
Urea mgm. N %..	L	—	7·10	8·10	31·00
„ U %......		—	15·01	17·01	65·10
mgm. N %......	U	—	7·23	7·85	30·00
„ U %......		—	15·12	16·59	63·00
Total Creatinine					
mgm. N %......	L	2·54	2·85	2·10	2·42
„ TC %.....		6·86	7·72	5·68	6·54
mgm. N %......	U	2·25	2·50	2·10	2·36
„ TC %.....		6·10	6·74	5·68	6·40
Uric acid mgm. N %......	L	0·34	0·39	0·36	0·52
„ UA %.....		1·01	1·18	1·07	1·56
mgm. N %......	U	0·20	0·20	0·16	—
„ UA %.....		0·60	0·60	0·48	—
Amino acid mgm. N %	L	7·12	5·60	6·63	10·69
	U	5·51	4·26	4·91	8·59
Rest Nitrogen mgm. N %...	L	14·27*	6·79	2·54	9·75
	U	9·66*	2·73	1·33	8·63†
Plasma......................		n.u.	n.u.	slightly icteric	icteric

* Includes "Urea Nitrogen." † Includes "Uric Acid Nitrogen."

Main Features of Analytical Data.

Hb.—Severe drop within eight days from 12–4 gm. per cent. 24 hours before death. No haemoglobinaemia noted; bilirubinaemia distinct.

N.P.N.—A marked increase from about 22 mgm. to 53 mgm. N per cent. in "laked" and from 17–50 mgm. in "unlaked" filtrate.

U.N.—An increase from 8–31 mgm. within 6 days.

U.A.N.—On day before death this fraction was highest but since only one figure is involved and the increase is not beyond the normal range, a definite correlation of this finding with the condition does not seem permissible.

A.A.N.—A high figure was found on the last day (± 11 mgm. N per cent.).

R.N.—Highest 24 hours before death.

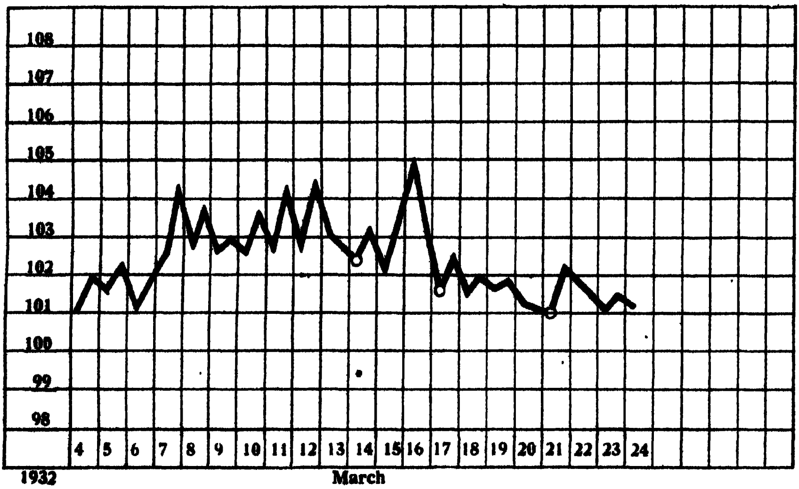
T.N.—Shows a drop from 2·9–1·7 gm. per cent. corresponding to the destruction of erythrocytes.

Sugar and ***T.C.N.***—Nothing unusual.

CASE IV.

B. 3053. *Anaplasmosis. Untreated—Recovered.*

Temperature Chart IV.



History.—B. 3053. An ox, born Armoedsvlakte, 12/2/29. Was inoculated against anthrax and blackquarter 22/10/30 and tested for immunity 12 months later. Drafted into the present experiment 29/1/32, being injected subcutaneously with 5 c.c. blood from B. 2889 (*vide*) on 11/2/32. From 7/3/32 to 23/3/32 anaplasms were found in only rare numbers in blood smears, and only slight anaemic changes developed. The temperature reaction was mild. The animal was kept under observation until 4/5/32.

TABLE 4.—*B.* 3053. *Anaplasmosis. Untreated—Recovered.*

Date *Time*		8/2/32. —	11/2/32. —	15/2/32. —	18/2/32. —	22/2/32. —	25/2/32. —	29/2/32. —	14/3/32. —	17/3/32. —	21/3/32. —
Temp. Reaction		N	P.I.N.	P.I.N.	P.I.N.	P.I.N.	P.I.N.	P.I.N.	R	R	N
Haemoglobin gm. %		18·20	17·51	15·42	18·57	16·87	17 18	15 98	15·19	16·29	14·28
Sugar mgm. %	L	—	49·00	53 48	49·00	43·43	48 10	47·20	47·40	43·86	49·70
	U	—	39·22	28·00	33 33	35·70	48·10	41·20	38·20	36·36	40·50
Total N. gm. %		3·304	3·346	2·214	3·430	3·263	3·290	3·445	3·130	3·340	3·032
N.P.N. mgm. %	L	22·37	21·28	25·64	21·88	22·71	21·48	22·71	17 00	21·13	20·00
	U	17·07	14·20	21·06	17·20	18·10	17·05	17·23	10·00	14·10	15·90
Coag. N. gm. N %	L	3·282	3·325	3·188	3 408	3·240	3·269	3·422	3·113	3·319	3·012
	U	3·287	3·332	3·193	3·413	3·245	3 273	3·428	3·120	3·326	3·016
Urea mgm. N %	L	6·17	4·48	7·70	8·70	9 60	8·20	8·10	4·60	5·25	6·63
,, U %		13·02	8·82	16·17	18·17	20·16	17·22	17·01	9·66	11·13	13·86
mgm. N %	U	5·52	4·26	7·80	6·70	9·10	7·00	8·10	4 40	5·72	6·23
,, U %		11·55	9·03	16·38	14·07	19·11	14·70	17·01	9·84	9·87	13·02
Total Creatinine											
mgm. N %	L	2·31	2·23	2·31	2·54	2·54	2 36	2 50	2·23	1·98	2·06
,, TC %		6·26	6·00	6·26	6 86	6·86	6·40	6·74	6·00	5·32	5·54
mgm. N %	U	1·91	1·91	1·88	2·36	2·48	2·00	2·42	2·10	1·67	1·84
,, TC %		5·14	5·14	5·06	6·40	6·70	5·40	6·54	5·68	4·50	4·96
Uric acid mgm. N %	L	0·63	0·61	0·69	0·57	0·52	0·58	0·54	0·40	0·49	0·37
,, UA %		1·88	1·83	2·07	1·70	1 55	1·74	1·62	1·21	1·48	1·12
mgm. N %	U	0·56	0·33	0·40	0·33	0·18	0·29	0·43	0·11	0·23	0·20
,, UA %		1·68	1·00	1·20	0·98	0·53	0 82	1·30	0·32	0·70	0·60
Amino acid mgm. N %	L	6·67	7·00	7·45	6·42	7·00	7·00	7·00	5·40	6·67	6·67
	U	4·86	4·83	6·67	5·00	4·30	6·36	4·24	2·90	4·12	3·90
Rest Nitrogen mgm. N %	L	6·59	6·96	7·19	3·65	3·05	3·34	4·36	4·37	6·74	4·27
	U	4·22	3·87	4·31	2·81	1·04	1·40	2·04	0·49	3·26	3·73
Plasma		n.u.	n.u.	n.u.	n.u.	n.u.	n.u.	n.u.	n.u.	n.u.	n.u.

Main Features of Analytical Data.

Hb.—Tendency for a decrease during the period of reaction. Only few parasites appeared and the temperature reaction, although undoubtedly present, was only slight.
U.A.N.—Shows the lowest level during the reaction ± 0·60— ± 0·45 mgm. N %. The drop is however, slight and not beyond the normal range encountered.
Sugar, N.P.N., U.N., T.C.N., A.A.N., R.N., T.N.—Nothing unusual.

Although undoubtedly a positive reaction, it must be regarded as an extremely mild one and one which, under ordinary conditions would escape detection. It is possible that this animal had at some previous time passed through an anaplasmosis attack and that the present reaction represents a slight breakdown of immunity due to injection of virulent blood from B. 2889 which reacted severely.

Case V.

B. 2308. *Anaplasmosis. Untreated—Recovered.*

No Temperature Chart.

History: B. 2308. A five-year old ox, in good condition, born on the station, 17/1/27. In December, 1931, it was immunised against anthrax and drafted on 29/1/32 into the anaplasmosis experiment, being injected subcutaneously on 11/2/32 with 5 c.c. blood from B. 2889 (*vide*). The animal was too wild to be temperatured, but frequent smear examinations revealed only a few parasites during the fifth week p.i., not associated with anaemic changes. No clinical symptoms were shown and this case must be regarded as a very mild reaction only, or even more probable an *abortive* reaction.

TABLE 5.—*B.* 2308. *Anaplasmosis (No temperature—too wild.)*

Date		8/2/32.	11/2/32.	15/2/32.	18/2/32.	22/2/32.	25/2/32.	29/2/32.	14/3/32.	17/3/32.	21/3/32.
Time		—	—	—	—	—	—	—	—	—	—
Temp. Reaction						Too wild.					
***Haemoglobin* gm. %**		18·57	17·18	17·16	19·33	18 22	17·28	18·20	20·60	20·18	13·87
***Sugar* mgm. %**	L	—	42·92	37·60	51·55	40·00	51·00	50·50	40·60	43·48	46·10
	U	—	36·76	38·80	34·01	29·60	45·80	43·90	32·50	36·90	41·15
***Total N.* gm. %**		3·480	3·416	3·444	3·598	3 648	3·507	3·578	3·606	3·662	3·487
***N.P.N.* mgm. %**	L	22·02	23·08	25·64	29·12	24·76	22·73	20·90	28·30	27·27	23·44
	U	16·13	13·53	17·48	21·00	18·75	16·70	14·10	16·30	20·36	16·63
***Coag. N.* gm. N %**	L	3·458	3·393	3·418	3·569	3·623	3·483	3·558	3·578	3·635	3·464
	U	3·464	3·399	3·427	3 577	3·629	3·490	3·564	3·590	3·642	3·410
***Urea* mgm. N %**	L	6·58	5·66	7·68	10·77	9·58	7·00	6·40	8·30	8·10	8·10
,, U %		13·86	11 97	16·17	22·68	21·16	14·70	13·44	17·43	17·01	17·01
mgm. N %	U	6·27	4·56	7·58	10·77	8·10	7·00	6·90	7·33	7·70	8·30
,, U %		13·23	9·66	15·96	22·68	17·01	14·70	12·39	15·33	16·17	18·43
Total Creatinine											
mgm. N %	L	2·31	2·31	2·13	2·54	2·96	2·74	2·50	2·66	2·29	2·31
,, TC %		6·26	6·26	5·76	6·86	8·00	7·40	6·74	7·20	6·20	6·26
mgm. N %	U	1·78	1·91	1·75	2·36	2 51	2·20	2·76	2·10	2·01	2·06
,, TC %		4·80	5·14	4·72	6·40	7·60	5·86	7·44	5·68	5·40	5·54
***Uric acid* mgm. N %**	L	0·56	0·63	0·71	0·51	0·58	0·57	0·54	0·48	0·56	0·42
,, UA %		1·68	1·88	2·13	1·54	1·67	1·70	1·62	1·44	1·68	1·21
mgm. N %	U	0·21	0 25	0·28	0·32	0·18	0·28	0 32	0·20	0·30	0·31
,, UA %		0·63	0·75	0 84	0 95	0·54	0·84	0·97	0 60	0·91	0·94
***Amino acid* mgm. N %**	L	6·60	6·67	7·00	7·00	8·24	6·93	7·00	7·00	7·78	7·51
	U	5·64	4·00	4·67	5·38	4 83	5·83	4·00	4·24	4·38	4·38
***Rest Nitrogen* mgm. N %**	L	5·97	7·81	8·12	8·30	3 40	4·49	3·56	10·86	9·55	5·26
	U	2·23	2·87	3·20	2·17	2 83	1 39	1·12	2·43	5·97	1·58
Plasma		n.u.	n.u.	n.u.	n.u.	n.u.	n.u.	n.u.	n.u.	n.u.	n.u.

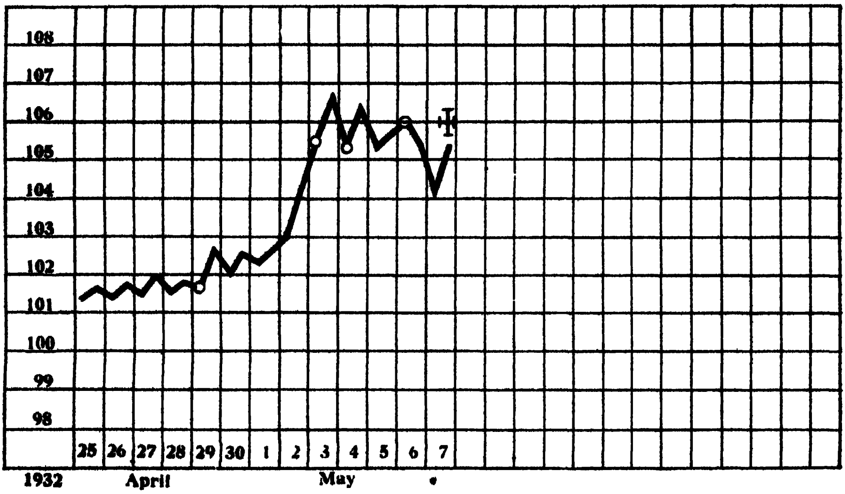
Main Features of Analytical Data.

The blood of this animal showed an *Anaplasma marginale* infection during the 4th and 5th week p.i., a temperature record being not obtainable owing to the animal's wildness. No marked anaemic changes were noted. The serial analyses for each constituent revealed no distinctive features before, during or after the time the marginal points were noted, and this case therefore represents a very mild reaction only. It is of interest to note the relatively very high level of the N.P.N. fraction.

CASE VI.

B. 3132. *Anaplasmosis. Untreated—Died,* 7/5/32.

Temperature Chart VI.



History : B. 3132. A two-year old Sussex heifer, born at Onderstepoort, 24/1/30. In good condition. Inoculated in November, 1930, against anthrax and blackquarter, the immunity against anthrax being tested 12 months later. Was drafted into this experiment on 6/4/32, being injected subcutaneously with 10 c.c. blood from B. 2889 (*vide*). The first anaplasms were seen on 25/4/32, i.e. the 20th day p.i. The temperature reaction set in on 1/5/32, 26 days after inoculation. The anaplasms became very numerous and marked anaemic changes in the blood developed. The animal ceased feeding, had a tucked up appearance, a staring coat, constipation, distinct icterus and severe anaemia. Died on 7/5/32 and post-mortemed at once. The following pathological-anatomical diagnosis was made : (P.M. No. 11037 of 7/5/32). Severe anaemia, generalised icterus, fatty changes and bile pigmentation of the liver and tumor splenis, findings which support the clinical diagnosis of anaplasmosis

TABLE 6.—*B.* 3132. *Anaplasmosis. Untreated—Died,* 7/5/32.

Date		15/4/32.	18/4/32.	21/4/32.	28/4/32.	3/5/32.	4/5/32.	6/5/32.
Time		11 a.m	—	—	—	—	—	—
Temp. Reaction		P.I.N.	P.I.N.	P.I.N.	P.I.N.	R.	R.	R.
Hb. gm. %		12·13	14·72	13·50	14·72	9·05	7·18	5·38
Sugar mgm. %	L	54·35	52·91	—	51·28	65·80	64·94	69·93
	U	40·65	39·22	—	37·45	57·47	61·00	64·94
Total Nitrogen gm. %		2·808	2·948	2·868	3·172	2·318	2·164	1·674
N.P.N. mgm. %	L	23·20	23·08	21·43	18·75	23·08	30·00	35·70
	U	17·82	16·66	15·04	13·04	18·18	25·00	30·50
Coag. N. gm. N %	L	2·785	2·925	2·847	3·153	2·295	2·134	1·638
	U	2·789	2·931	2·853	3·159	2·300	2·139	1·644
Urea mgm. N %	L	9·00	7·16	7·81	6·40	12·16	15·67	18·30
„ U %		18·90	15·12	16·38	13·44	25·62	33·60	38·43
mgm. N %	U	9·00	7·00	7·51	6·07	11·95	15·00	18·08
„ U %		19·90	14·70	15·75	13·81	25·20	31·50	38·00
Total Creatinine								
mgm. N %	L	2·66	2·33	1·78	2·23	2·85	2·29	2·36
„ TC %		7·20	6·26	4·80	6·00	7·72	6·16	6·36
mgm. N %	U	2·23	2·04	1·49	1·89	2·29	2·23	1·89
„ TC %		6·00	5·54	4·00	5·14	6·16	6·00	5·14
Uric Acid mgm. N %	L	0·73	0·36	0·52	0·50	0·32	0·27	0·32
„ UA %		2·19	1·07	1·56	1·49	0·97	0·80	0·97
mgm. N %	U	0·35	0·13	0·25	0·23	0·19	0·16	0·27
„ UA %		1·05	0·40	0·75	0·70	0·57	0·48	0·81
Amino acid mgm. N %	L	6·36	7·00	6·40	6·36	5·83	5·83	5·83
	U	4·24	3·90	3·68	3·68	3·18	4·38	4·52
Rest Nitrogen mgm. N %	L	4·45	6·23	4·92	3·26	1·88	5·94	8·89
	U	2·00	3·62	2·11	1·17	0·57	3·23	5·73
Plasma		n.u.	n.u.	n.u.	n.u.	icteric	—	—

Main Features of Analytical Data.

Hb.—Shows a marked decrease from 14–5·3 gm. % 24 hours prior to death; this drop supervening within eight days. No haemoglobinaemia, but bilirubinaemia was noticeable.

Sugar.—Showed a slight tendency towards an increase in both filtrates.

N.P.N.—Increased from ± 20–36 mgm. N % and from ± 17–30·50 mgm. N % in "laked" and "unlaked" filtrates respectively.

U.N.—A definite increase from 8–18 mgm. N % is noted.

U.A.N.—Shows a tendency to decrease in both filtrates.

R.N.—An increase is observed shortly before death.

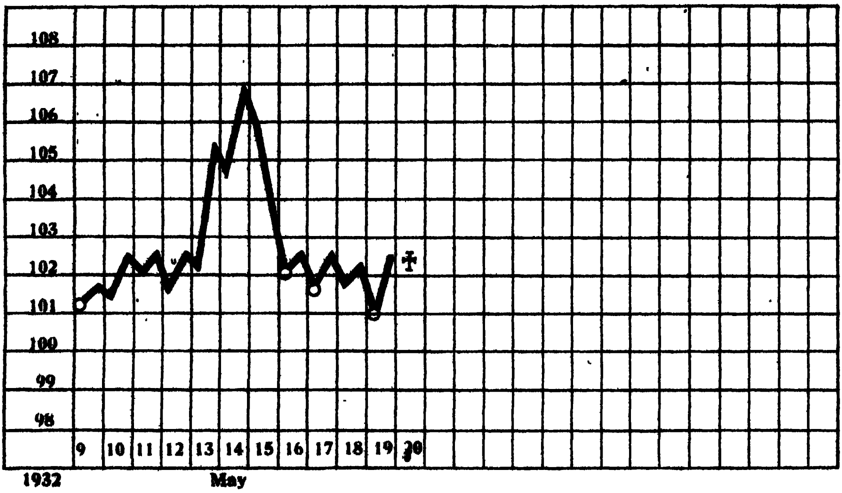
T.N.—Drops from 2·8–1·7 gm. N % paralleling the decline in Hb. content.

T.C.N., ***A.A.N.***—Nothing unusual.

CASE VII.

B. 2269. *Anaplasmosis. Treated—Died,* 20/5/32.

Temperature Chart VII.



History: B. 2269. A five-year-old cross-bred ox in fair condition. Immunised against anthrax, 4/12/31 and drafted into anaplasmosis experiment 6/4/32, on which date it was injected with 10 c.c. blood from B. 3053 (*vide*). Anaplasms were first noted the 30th day p.i. and the temperature rose on the 38th day p.i. The parasites became numerous and anaemic blood changes developed. The animal ceased feeding, developed constipation, salivation and icterus, and died on the 44th day p.i. Received an injection on the 14th and 19th May.

At post-mortem (P.M. No. 11128 of 20/5/32) the following was found: generalised icterus, anaemia, cholaemia, slight oedema and emphysema of the lungs, acute tumor splenis, tumor hepatis and pigmentation. A haemoglobinuria was also noted (sequel therapeutic injection ?).

TABLE 7.—*B*. 2269. *Anaplasmosis. Treated—Died,* 7.30 *a.m. on* 20/5/32.

Date......................... *Time*.........................		12/4/32. 11 a.m.	14/4/32. 12 a.m.	18/4/32. —	21/4/32. —	28/4/32. —	4/5/32. —	9/5/32. —	16/5/32. —	17/5/32. —	19/5/32. —
Temp. Reaction................		P.I.N.	P.I.N.	P.I.N.	P.I.N.	P.I.N.	P.I.N.	P.I.N.	R.	R.	R.
Haemoglobin gm. %............		14·49	18·53	19·73	18·57	23·18	18·20	21·18	15·42	12·42	6·44
Sugar mgm. %............	L	78·74	46·95	66·30	51·55	47·17	43·17	53·76	65·52	61·35	30·58
	U	66·00	—	38·33	35·09	36·00	31·45	42·37	55·87	51·80	26·04
Total Nitrogen gm. %.........		3·242	3·564	3·548	3·648	3·774	3·690	3·522	2·948	2·780	2·024
N.P.N. mgm. %..........	L	25·00	33·33	25·27	23·08	22·06	21·43	24·50	30·94	28·60	51·38
	U	18·75	25·00	14·71	15·43	18·73	15·80	18·46	23·10	21·00	45·12
Coag. N. gm. N %........	L	3·217	3·531	3·523	3·625	3·752	3·669	3·498	2·917	2·751	1·973
	U	3·221	3·539	3·533	3·633	3·755	3·674	3·504	2·925	2·759	1·979
Urea mgm. N %..........	L	11·50	11·50	7·62	9·25	8·75	6·69	9·52	15·62	10·24	19·20
,, U %..........		24·15	24·15	15·96	19·53	18·48	14·07	20·00	32·76	21·72	40·32
mgm. N %..........	U	11·12	10·90	7·10	8·52	9·06	6·02	8·52	14·62	10·00	18·61
,, U %..........		23·31	22·89	15·00	17·85	18·90	12·60	17·85	30·66	21·00	39·06
Total Creatinine											
mgm. N %.....	L	2·23	2·31	2·23	1·97	2·23	2·11	2·11	2·35	3·08	2·42
,, TC %....		6·00	6·26	6·00	5·32	6·00	5·68	5·68	6·36	8·30	6·54
mgm. N %.....	U	2·06	2·96	1·90	1·67	1·82	1·67	1·75	1·82	2·27	2·17
,, TC %....		5·54	8·00	5·14	4·50	4·90	4·50	4·70	4·90	6·16	5·84
Uric acid mgm. N %.....	L	0·30	0·42	0·33	0·71	0·45	0·37	0·40	0·37	0·37	0·47
,, UA %....		0·90	1·25	1·00	2·14	1·36	1·10	1·20	1·10	1·10	1·41
mgm. N %.....	U	0·21	0·15	0·20	0·50	0·32	0·16	0·22	0·71	0·19	0·36
,, UA %....		0·64	0·45	0·59	1·50	0·96	0·49	0·67	0·52	0·57	1·09
Amino acid mgm. N %....	L	7·78	6·67	7·87	7·57	7·00	8·24	7·78	8·24	8·24	14·58
	U	4·24	4·12	3·50	3·40	4·12	3·90	3·68	4·03	5·11	11·66
Rest Nitrogen mgm. N %...	L	3·19	12·07	7·22	3·58	3·63	4·02	4·69	4·36	6·67	14·71
	U	1·12	6·87	2·02	1·34	3·47	4·05	3·29	2·46	3·44	12·38
Plasma......................		n.u.	n.u.	n.u.	n.u.	n.u.	n.u.	n.u.	icteric	icteric	icteric

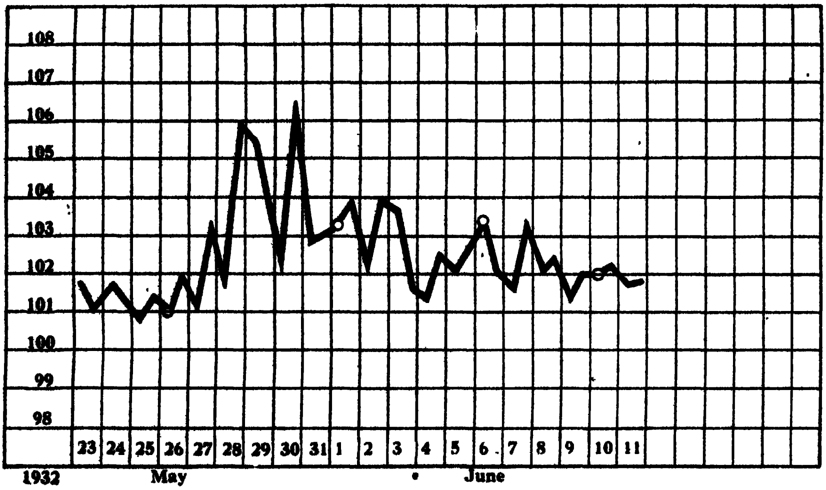
Main Features of Analytical Data.

Hb.—A marked decrease from ± 20–6 gm. % is noted. Towards death the drop is very severe, the Hb. level dropping within 48 hours from 12–6 gm. % 24 hours before death.
N.P.N.—Shows a sharp rise in both filtrates from ± 24–57 mgm. N % and from ± 18–45 mgm. N % in "laked" and "unlaked" filtrates respectively.
U.N.—Rises from ± 8–19 mgm. N % in both filtrates, the increase being particularly striking 24 hours before death.
A.A.N.—Increases considerably in both filtrates shortly before death.
R.N.—Here also an increase can be recorded from ± 5–15 mgm. N % and from ± 4–12 mgm. N % in "laked" and "unlaked."
Sugar and T.C.N.—Nothing unusual.

CASE VIII.

B. 3198. *Anaplasmosis. Treated—Recovered.*

Temperature Chart VIII.



History: B. 3198. A two-year-old heifer (born at Onderstepoort on 5/3/30), in good condition. Was inoculated against anthrax and blackquarter in November, 1930, the anthrax immunity being tested 12 months later. It was then drafted into the anaplasmosis experiment on 6/5/32, receiving 10 c.c. blood from bovine 3132 (*vide*) subcutaneously. Frequent smear examinations were made. On 21/5/32, i.e. 15 days p.i., the first *Anaplasma marginale* appeared. Before the temperature rose, the parasites were fairly frequent, and anisocytosis was noted, and by 1/6/32 marked blood changes, e.g. basophilia, polychromasia, normoblasts had developed. The parasites in rare numbers continued to be present for 5–6 weeks, but the blood changes showed a continuous improvement, so that by 7/7/32 only a slight anisocytosis remained. During the reaction the animal ceased feeding, was dull, showed icterus and passed only small amounts of rather firm faeces. A chemotherapeutic injection was given during the main reaction (30/5/32).

TABLE 8.—*B.* 3198. *Anaplasmosis. Treated—Recovered.*

Date *Time*		9/5/32. —	13/5/32. —	17/5/32. —	26/5/32. —	1/6/32. —	6/6/32. —	10/6/32. —	14/6/32. —	20/6/32. 10.30 a.m.	24/6/32. —
Temp. Reaction		P.I.N.	P.I.N.	P.I.N.	P.I.N.	R.	R.	R.	N.	N.	N.
Haemoglobin gm. %		15·42	14 95	13·87	11·53	4·91	4·45	5·59	7·66	10·35	10·99
Sugar mgm. %	L	59·88	60·61	61·35	61·35	71·94	80·00	—	—	—	52·36
	U	51·28	46·08	53·19	52·91	66·67	64·10	—	—	—	45·66
Total N. gm. N %		3·032	2·941	2·814	2·612	1·730	1·611	1·884	2·206	2·360	2·339
N.P.N. mgm. %	L	25·00	21·43	17·65	13·63	27·71	18·75	14·85	15·39	13·40	13·04
	U	17·27	15·04	12·00	10·00	23·08	15·00	10·71	9·75	9·40	9·84
Coag. N. gm. N %	L	3·007	2·920	2·796	2·598	1·702	1·592	1·869	2·191	2·347	2·326
	U	3·015	2·926	2·802	2·602	1·707	1·596	1·873	2·196	2·351	2·329
Urea mgm. N %	L	9·00	8·09	4·63	3·50	15·26	7·85	4·48	3·41	3·65	3·08
,, U %		18·90	17·01	9·66	7·35	32·13	16·59	9·45	7·14	7·77	6·51
mgm. N %	U	8·09	7·97	4·45	3·30	15 00	7·66	4·41	3·20	3·76	3·08
,, U %		17 01	16·80	9 45	6·93	31·50	16·17	9·24	6·72	7·98	6·51
Total Creatinine mgm. N %	L	2·23	1·87	2·66	2 01	2·35	1·71	—	—	—	2·04
,, TC %		6·00	5·02	7·20	5·40	6·36	4·60	—	—	—	5·50
mgm. N %	U	1·98	1·61	2·17	1·64	1·67	1·54	—	—	—	1·75
,, TC %		5·32	4·32	5·84	4·40	4·50	4·16	—	—	—	4·70
Uric acid mgm. N %	L	0·44	0·42	0·46	0·39	0·32	0·26	—	—	—	0·36
,, UA %		1·31	1·25	1·38	1·18	0·97	0·78	—	—	—	1·07
mgm. N %	U	0·17	0·18	0·19	0·18	0·22	0·20	—	—	—	0·18
,, UA %		0·50	0·55	0·56	0·53	0·67	0·60	—	—	—	0·54
Amino acid mgm. N %	L	6·86	6·28	6·36	5·00	6 14	4·49	4·38	5·17	4·02	4·67
	U	3·50	3·50	3 33	3·11	3·68	2·92	2·72	2·92	3·15	2·72
Rest Nitrogen gm. %	L	6·47	4·77	3·54	2·73	3·64	4·34	5·99*	6·78*	5·73*	2·89
	U	3·53	1·78	1·86	1·77	2·51	2·68	3·58*	3·63*	2·59*	2·11

* Includes "Total Creatinine Nitrogen" and "Uric Acid Nitrogen."

Main Features of Analytical Data.

Hb.—This shows a decrease commencing before the actual temperature reaction set in, the Hb. falling from 15–4·45 gm. % over a period of about three weeks, an improvement setting in as soon as the temperature has reached normal.

Sugar.—Shows a tendency towards a slight increase.

N.P.N. and U.N.—Both these fractions increase during the actual temperature reaction, dropping to the normal level before even the hyperthermia has ended.

U.A.N.—Shows a decrease during the reaction, the return to normal being prolonged for a considerable period.

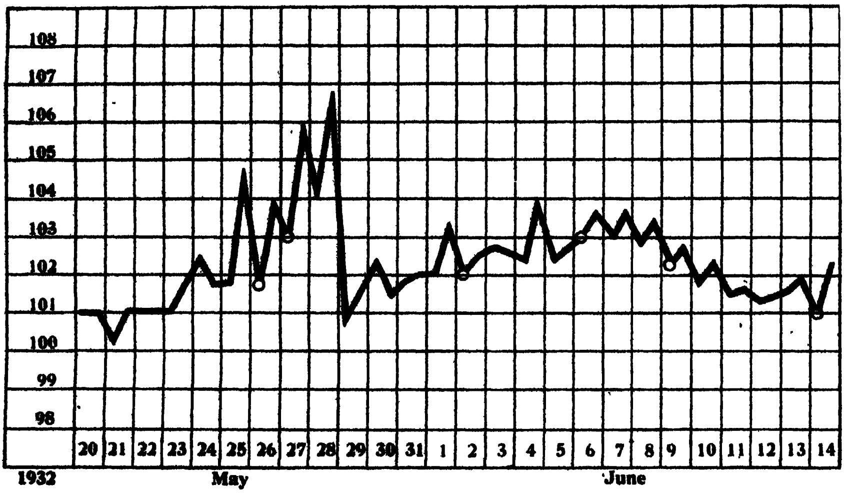
T.N.—A decrease from 3·0–1·6 gm. % can be recorded during the course of the condition, recovery being somewhat retarded.

T.C.N. and A.A.N.—Nothing unusual.

CASE IX.

B. 3126. *Anaplasmosis. Treated—Recovered.*

Temperature Chart IX.



History: B. 3126. A two year old tolly in good condition (born Armoedsvlakte, 17/1/30). Immunised against blackquarter and anthrax in November, 1930, anthrax immunity being tested 12 months later. Placed into anaplasmosis experiment on 6/5/32, being injected subcutaneously with 10 c.c. blood from B. 3132 (*vide*). Fifteen days later the first anaplasms appeared in the blood, the temperature rising on the 20th day p.i. The anaplasms became increasingly numerous and severe anaemic changes including anisocytosis, polychromasia, basophilia, and Jolly bodies developed. The animal showed clinically loss of appetite, dullness, slight icterus and constipation. Chemotherapeutic treatment was undertaken on the 20th and 30th May, and the 2nd June with apparently favourable results, the parasites becoming markedly decreased in number. The anaemic changes in the blood gradually disappeared, only a slight anisocytosis persisting for another few weeks. The animal made an uneventful recovery and was discharged on 17/7/32.

TABLE 9.—*B.* 3126. *Anaplasmosis.* *Treated—Recovered.*

		9/5/32.	13/5/32.	17/5/32.	26/5/32.	27/5/32.	2/6/32.	6/6/32.	9/6/32.	14/6/32.	20/6/32.	24/6/32.	1/7/32.	8/7/32.	14/7/32.
Time		—	—	—	—	—	7.15 a.m.	—	—	—	—	—	—	—	—
Temp. Reaction		P.I.N.	P.I.N.	P.I.N.	R.	R.	R.	R.	R.	R.	N.	N.	N.	N.	N.
***Haemoglobin* gm. %**		20·60	20·18	18·20	13·12	11·39	4·91	4·74	5·32	8·14	11·39	12·42	14·28	14·95	15·19
***Sugar* mgm. %**	L	52·63	52·36	49·02	58·82	59·17	75·19	86·21	79·36	—	—	49·02	49·75	54·95	53·48
	U	44·44	41·32	39·68	46·30	53·48	68·97	74·63	74·63	—	—	43·86	46·87	42·37	43·10
***Total N.* gm. N %**		3·550	3·480	3·382	2·990	2·675	1·820	1·814	1·842	2·248	2·717	2·892	3·011	2·976	3·193
***N.P.N.* mgm. %**	L	27·27	22·22	21·43	20·00	20·00	20·06	19·54	17·88	15·79	15·25	15·85	14·92	12·40	15·75
	U	17·15	15·72	15·28	15·08	15·00	17·97	14·28	12·50	16·00	8·57	12·20	11·77	10·00	10·10
***Coag. N.* gm. N %**	L	3·523	3·458	3·361	2·970	2·655	1·800	1·794	1·824	2·232	2·702	2·876	2·996	2·964	3·176
	U	3·533	3·564	3·367	2·975	2·660	1·802	1·800	1·830	2·238	2·708	2·880	2·999	2·966	3·183
***Urea* mgm. N %**	L	9·00	8·66	8·05	8·17	8·52	9·00	7·47	5·97	4·68	3·00	5·78	4·26	3·76	3·88
„ U %		18·90	18·17	17·00	17·22	17·85	18·90	15·33	12·60	9·87	6·30	12·18	9·03	8·00	8·19
mgm. N %	U	8·30	8·09	7·51	7·51	8·52	9·52	7·12	5·92	4·13	2·92	5·49	4·06	3·65	3·65
„ U %		17·43	17·00	15·75	15·75	17·85	20·00	15·00	12·39	8·61	6·09	11·55	8·71	7·56	7·56
Total Creatinine															
mgm. N %	L	2·04	2·04	2·96	2·35	2·42	2·57	2·34	2·23	—	—	2·50	2·34	2·17	2·23
„ TC %		5·50	5·50	8·00	6·36	6·54	6·96	6·36	6·00	—	—	6·74	6·36	5·84	6·00
mgm. N %	U	1·91	1·61	2·57	1·82	2·10	2·01	1·82	1·49	—	—	1·98	1·75	1·71	2·01
„ TC %		5·14	4·32	6·96	4·90	5·68	5·40	4·90	4·00	—	—	5·32	4·70	4·60	5·40
***Uric acid* mgm. N %**	L	0·33	0·37	0·38	0·33	0·29	0·25	0·25	0·30	—	—	0·29	—	0·31	0·38
„ UA %		0·99	1·02	1·14	0·98	0·87	0·74	0·74	0·89	—	—	0·87	—	0·94	1·07
mgm. N %	U	0·13	0·16	0·14	0·16	0·13	0·15	0·19	0·28	—	—	0·16	—	0·18	0·18
„ UA %		0·40	0·48	0·42	0·47	0·40	0·44	0·57	0·84	—	—	0·48	—	0·55	0·53
***Amino acid* mgm. N %**	L	7·78	6·80	6·67	5·83	5·69	5·60	5·17	4·93	4·61	4·67	4·52	4·93	4·58	4·67
	U	3·68	3·68	3·18	3·68	3·18	3·50	3·41	2·72	2·60	2·72	2·92	3·50	2·50	2·72
***Rest Nitrogen* mgm. N %**	L	8·12	4·35	3·37	3·32	3·08	2·58	4·31	4·45	6·50*	7·58*	2·76	3·39†	3·58	4·54
	U	3·13	2·18	1·88	1·91	1·07	2·79	1·74	2·09	3·27*	2·93*	1·65	2·46†	1·96	1·54
Plasma		n.u.	n.u.	n.u.	icteric	icteric	icteric	icteric	n.u.	n.u.	n.u.	n.u.	n.u.	n.u.	n.u.

* Includes "Total Creatinine Nitrogen" and "Uric Acid Nitrogen." † Includes "Uric Acid Nitrogen."

Main Features of Analytical Data.

Hb.—Drops from 20–4·7 gm. % over a period of three weeks, and returns to the normal level setting in before the temperature has dropped. Haemoglobinaemia absent, bilirubinaemia present.

Sugar.—Tendency to increase.

N.P.N. and U.N.—An interesting feature of these two N. fractions in this case is that no significant changes in amount occur, in spite of the relatively heavy drop in Hb. content, and severe morphological blood changes.

T.C.N., U.N.N., A.A.N., and R.N.—Nothing unusual.

T.N.—Decreases from 3·5–1·8 gm. N %, the lowest level being reached when the Hb. level is at its lowest.

(*d*) THE NORMAL RANGE OF VALUES OBTAINED FOR Hb., SUGAR, N.P.N., U.N., T.C.N., U.A.N., A.A.N., R.N. AND T.N. IN BOVINE BLOOD.

Before it is possible to determine what represents abnormal concentrations of any particular constituent, the normal variations, preferably determined under the same environmental conditions, must be known. Fragmentary researches in this connection exist for most or all of the domestic animals but no complete range of data comprising a relatively large number of animals of different breeds and ages of one species on a fixed diet and representing analyses taken over a period of 12–18 months to note seasonal variations, effect of pregnancy, diet, etc., exists. A further complication arises out of the fact that with different methods different results are obtained, the variations in some cases being considerable, and comparisons of results obtained by different workers becomes difficult or impossible. Analyses taken over short periods, or worse still, representing one single analysis, are of little value, since the concentrations of various constituents may vary considerably—even on a fixed diet—during the course of a year, due to ageing of the animal and seasonal variation. This is well brought out in a series of analyses made on sheep blood by Hamersma (1933) over a period of about twelve months. The same tendencies can be observed in some of the serial analytical figures submitted here, particularly in those *bovines* for which the data collected extends over ten weeks.

The " normal " figures utilised here represent 50 60 analyses of " normal " bloods from animals of various breeds and ages and made during different times of the year. Some of the date are from animals not tabulated here, but which were intended for infection especially in connection with redwater experiments, but which proved refractory. In each case the minimum and maximum range is given; the " average " obtained by adding all the normal data and dividing by the respective number of analyses, and tables to indicate the grouping of data. The percentage variation of constituents in the " laked " and " unlaked " filtrates respectively, is also given, together with the " average " percentage variation. The latter has primarily been given for the purpose of obtaining " factors " which would enable figures obtained from " laked " filtrate analyses being recalculated in terms of " unlaked " filtrates and *vice versa*, and thus form some basis for comparison of analytical data gathered by different research workers using either filtrate.

Haemoglobin.

Minimum-maximum range: 12·1–23·2 gm. Hb. %.

"Average " Hb. content: 17·1 gm. Hb. %.

Grouping according to distribution.

gm. Hb. %.	*Occurrence.*	*gm. Hb. %.*	*Occurrence.*
11–12	0	17–18	7
12–13	2	18–19	9
13–14	2	19–20	2
14–15	7	20–21	4
15–16	5	21–22	1
16–17	3	22–23	1

Sugar.

Minimum-maximum variation " laked " 37·6–95 mgm. %.

"Average laked," 60 mgm. %.

Mininum-maximum variation " unlaked," 31·5–82·6 mgm. %.

Average " unlaked," 47·8 mgm. %.

Percentage difference between "laked" and "unlaked" filtrates range from 4–27 % with an average variation of 20 %, the "laked" being the higher.

Grouping according to distribution.

"*Laked.*"		"*Unlaked.*"	
mgm. %.	*Occurrence.*	*mgm.* %.	*Occurrence.*
35–45	7	30–40	15
45–55	17	40–50	12
55–65	7	50–60	6
65–75	4	60–70	5
75–85	2	70–80	1
85–95	3	80–90	1

Total Nitrogen.

Minimum-maximum variation, 2·5–3·5 gm. N %.

Average, 3 gm. N %.

Grouping according to distribution.

gm. N %.	*Occurrence.*
2·5–2·7	8
2·7–2·9	7
2·9–3·1	4
3·1–3·3	7
3·3–3·5	6

Non-Protein Nitrogen.

Minimum-maximum variation "laked," 12·5–25 mgm. N %.

Average "laked," 18·2 mgm. N %.

Minimum-maximum variation "unlaked," 9·4–18·7 mgm. N %.

Average "unlaked," 13·5 mgm. N %.

Percentage differences between "laked" and "unlaked" filtrates range from 12·7–36·2 % with an average of 20·3 %: the "laked" concentration being always higher.

Grouping according to distribution.

"*Laked.*"		"*Unlaked.*"	
mgm. N %.	*Occurrence.*	*mgm. N* %.	*Occurrence.*
12–14	4	Under 11	0
14–16	6	9–11	9
16–18	9	11–13	9
18–20	11	13–15	10
20–22	4	15–17	7
22–24	4	17–19	5
24–26	2	Over 19	0

Urea Nitrogen.

Owing to the only small difference between the "laked" and "unlaked" U.N. concentration no differentiation is made between the two filtrates. The "unlaked" U.N. is in the vast majority of cases slightly lower.

Minimum-maximum variation, 3·0–9·0 mgm. N %.

Average, 5·7 mgm. N %.

Grouping according to distribution.

mgm. N %.	*Occurrence.*	*mgm. N %.*	*Occurrece.*
3–4	12	6–7	16
4–5	12	7–8	3
5–6	12	8–9	5

Total Creatinine Nitrogen.

Minimum-maximum variation "laked," 1·9-2·9 mgm. N %.

Average "laked," 2·3 mgm. N %.

Minimum-maximum variation "unlaked," 1·4–2·6 mgm. N %.

Average "unlaked," 1·9 mgm. N %.

Percentage difference between "laked" and "unlaked" filtrates vary from 3–30 % with an average variation of 14 %, the "laked" being the higher.

Grouping according to distribution.

"Laked.		*"Unlaked."*	
mgm. N %.	*Occurrence.*	*mgm. N %.*	*Occurrence.*
1·9–2·1	9	1·4–1·6	2
2·1–2·3	13	1·6–1·8	10
2·3–2·5	3	1·8–2·0	8
2·5–2·7	3	2·0–2·2	3
2·7–2·9	2	2·2–2·4	5
—	—	2·4–2·6	2

Uric Acid Nitrogen.

Minimum-maximum variation "laked," ·33–·73 mgm. N %.

Average "laked," ·46 mgm. N %.

Minimum-Maximum variation "unlaked," ·14–·56 mgm. N %.

Average "unlaked," ·23 mgm. N %.

Percentage difference between "laked" and "unlaked" filtrates vary from 9–63 % with an average of 50 %, the "laked" being the higher.

Grouping according to distribution.

"Laked."		*"Unlaked."*	
mgm. N %.	*Occurrence.*	*mgm. N %.*	*Occurrence.*
·33–·43	16	·14–·24	22
·43–·53	6	·24–·34	5
·53–·63	5	·34–·44	2
·63–·73	3	·44–·56	1

Amino Acid Nitrogen.

Minimum-maximum variation "laked," 4·3–8·4 mgm. N %.

Average "laked," 5·8 mgm. N %.

Mininum-maximum variation "unlaked," 2·1–5·6 mgm. N %.

Average "unlaked," 3·8 mgm. N %.

Percentage differences between "laked" and "unlaked" filtrates vary from 14–50 % with an average of 34·6 %, the "laked" being the higher.

Grouping according to distribution.

"*Laked.*" mgm. N %.	Occurrence.	"*Unlaked.*" mgm. N %.	Occurrence.
4·3–5·4	15	2·1–3·1	10
5·4–6·4	5	3·1–4·1	10
6·5–7·4	8	4·1–5·1	4
7·4–8·4	2	5·1–6·1	6

Rest Nitrogen.

Minimum-maximum variation "laked," 2·0–6·6 mgm. N %.

Average "laked," 4·0 mgm. N %.

Minimum-maximum variation "unlaked," 1·4–4·2 mgm. N %.

Average "unlaked," 2·1 mgm. N %.

Percentage differences between "laked" and "unlaked" filtrates vary from 0–72 % with an average of 44 %, the "laked" being always the higher.

Grouping according to distribution.

"*Laked.*" mgm. N %.	Occurrence.	"*Unlaked.*" mgm. N %.	Occurrence.
2·0–2·5	2	1·4–2·0	11
2·5–3·0	4	2·0–2·5	6
3·0–4·5	7	2·5–3·0	1
4·5–5·5	5	3·0–3·5	2
5·5–6·0	0	3·5–4·0	0
6·0–6·6	2	4·0–4·5	1

(*e*) GENERAL SUMMARY OF PATHOLOGICAL FINDINGS.

As was to be expected with a disease of the nature of anaplasmosis several well marked features emerge from the mass of data collected. The intensity or the degree of the changes vary from practically nil in the case of mild attacks to very marked in fatal cases, with intermediate ranges for recovery cases. The strain of *Anaplasma marginale*, which was used for all the subinoculations, was a virulent one, as is evinced by the short incubation period noted in some of the cases, the severe reactions which in three cases out of nine proved fatal and threatened with extinction several others. On the other hand, there were two very mild cases in which the only symptoms were a slight febrile reaction and the presence of a few anaplasms in the blood smears. This may have been due to the individual resistence to infection varying, or that these two animals had previously had anaplasmosis and that the reaction here merely represented a yartial breakdown of immunity as a result of a heavy infection with a virulent strain. In this summary attention will be focussed on those constituents specially influenced as a sequence to this infection.

Haemoglobin.

This constituent of the blood shows the most noticeable alterations in amount in the direction of a decrease. In some instances the beginning of the destruction of erythrocytes would coincide with the febrile reaction, in others the decrease of the Hb. level could be noticed considerably before then. This is particularly well exemplified in Case VIII, where the Hb. had dropped from 15–11 gm. per cent. over a period of two weeks before the hyperthermia had set in.

The time interval till the minimum Hb. level is reached fluctuates considerably, e.g. in Case 1, 18 days; in Case III, three weeks: in Case III, six days; in Case VI, eight days: in Case VII, ten days; in Case VIII, four weeks; and in Case IX, three weeks.

There is a distinct acceleration of destruction noted in several cases, the percentage destruction of erythrocytes per unit of time increasing towards the acme of the temperature record. Case VII shows this especially well, the Hb. content dropping from 21–12 gm. per cent. in eight days, but thereafter from 12–4 gm. per cent. within 48 hours. The mechanism of erythrolysis (or erythrorhexis) in anaplasmosis in unknown—whether it is a simple rupture of the stroma due to the penetration of the parasite into or out of the red cell, or whether an erythrolytic toxin is produced, is unknown. A large number of infected cells may be retained in the spleen and destroyed. The increased rate of destruction, however, could be explained on the normal acceleration of multiplication of the parasite. Compared with an acute redwater infection, however, erythrolysis is comparatively slow in anaplasmosis, normally no such amounts of Hb. being liberated into the plasma as to cause haemoglobinuria. The Hb. liberated is converted into bile pigments and partly excreted through the liver and urine, and partly absorbed by the tissues, giving rise to the phenomenon of icterus.

Sugar.

In cases I, II, VII, VIII, IX there exists a tendency for a slight temporary increase in the sugar concentration during the fever reaction. Case VIII shows an initial slight rise succeeded by a drop 24 hours before death.

Non-Protein Nitrogen.

The conditions found here vary according to the severity of the reaction, a marked increase was noted in all the three fatal cases (III, VI and VII), and in the severe reaction in Case II. In case III the increase towards the end was from ± 20 mgm. to 53 mgm. N per cent. and from 14–49 mgm. N per cent. in "laked" and "unlaked" respectively. In the other fatal cases VI and VII, it ranged up to 37 mgm. N per cent. and in Case II up to ± 25 mgm. N per cent. The increase only occurred during the actual febrile reaction and represents chiefly the increased U.N. Case VIII showed only a slight upward tendency, the others not accounted for remaining normal, although they were definite positive, even if mild reactors.

Urea Nitrogen.

This fraction shows the identical features as the N.P.N. and is primarily responsible for the increases mentioned above. In Case II a rise from ± 5– ±10 mgm. per cent. is found, in Case III ± 8– ± 31 mgm. N percent., in Case VI and VII ± 8–18 mgm. N per cent. In the others no increase or only a very slight temporary one is found. The increase is only met with during the temperature reaction, and various suggestions as to its probable origin arise. e.g. amongst others it may be mentioned that the increased metabolism associated with any rise in body temperature may be responsible; or it may be associated with protein catabolism in the circulating blood due to enzymes liberated by the protozoan; or may represent a metabolic excretion product of protozoan activity: on the other hand, the fatty degeneration of the kidneys found in severe cases of anaplasmosis, may lead to a retention as a result of renal dysfunction. Sufficient data for an explanation is as yet not available, but this fact emerges that an increase in U.N. above 15 mgm. N per cent. should be regarded as unfavourable from a prognostic point of view.

Total Creatinine Nitrogen.

In the severe cases a slight temporary rise can be observed: in all the other cases nothing. The range, however, does not exceed the normal limits of variations and cannot, therefore, be regarded of any special significance.

Uric Acid Nitrogen.

In the majority of cases a definite decrease in this fraction can be recorded, coinciding with the hyperthermia. A notable exception, however, is one fatal case in which the U.A.N. remained normal but increased 24 hours before death.

Amino-Acid Nitrogen.

Except in the fatal cases where a marked increase is noted before death, no definite change in either direction is observable. The increase in fatal cases is recorded only before death and figures up to 14·6 and 11·6 for "laked" and "unlaked" filtrates respectively. Although several explanations are conceivable, the assumption of hepatic dysfunction due to the severe strain thrown on this organ through the increased bile secretion, is favoured as the cause.

Rest Nitrogen.

A definite increase up to 14 mgm. N per cent. is only noted in the fatal cases shortly before death. The R.N. represents here probably the decomposition or metabolic products of the protozoan, and a closer investigation of this fraction should be of great interest.

Total Nitrogen.

Marked decreases, corresponding to the drop in haemoglobin, are encountered in all the severe or fatal cases, the degree of diminution depending primarily on the severity of the erythrolysis.

(*f*) CONCLUSION.

Marked blood changes are recorded in anaplasmosis, practically every constituent being affected. The Hb. and T.N. contents drop severely, the U.A.N. also decreasing. On the other hand, striking increases are noted in the N.P.N. and the U.N., with less marked though well defined increases in the sugar level. The A.A.N. and R.N. increase shortly before death in fatal cases.

III. PIROPLASMOSIS OF CATTLE (TEXAS FEVER, REDWATER).

The piroplasmosis referred to here is the infection obtained with *Piroplasma bigeminum.* In connection with these researches, seven bovines were utilised, and after several analyses of the normal blood had been performed in each case, they were injected with infective blood. In only three cases was a reaction obtained, but with the exception of the one case given here, they were so mild, that they could be regarded as virtually non-reactors. An occasional rare parasite was found in the blood smears, and a slight undulation in the temperature record. Analyses during this period showed slight blood changes which were in the same general direction as those recorded here in Table I. It was then decided to repeat this experiment in the near future with a larger number of animals, and only to submit in the meantime the data in respect of the one typical severe case recorded here, primarily for comparison and contrast with the figures obtained in connection with anaplasmosis.

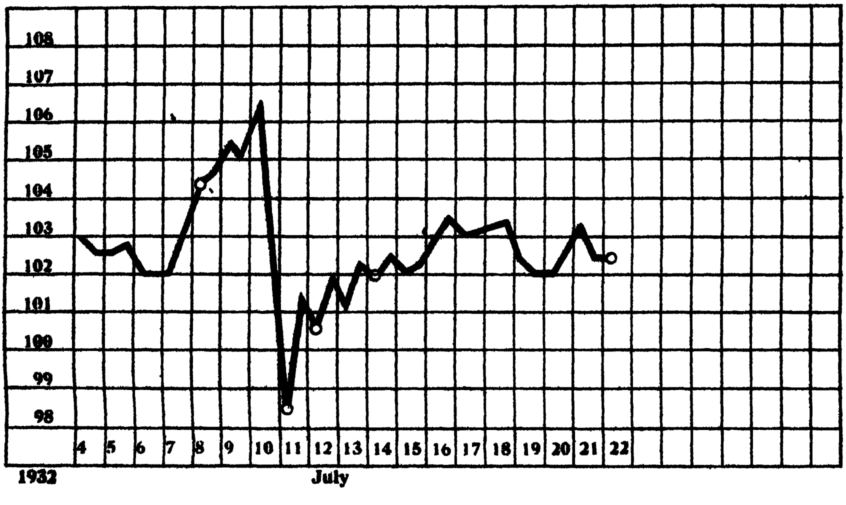
In a severe case of piroplasmosis the destruction of erythrocytes is so rapid that the liberated haemoglobin is partly excreted as such through the kidneys, resulting in haemoglobinuria, partly converted into bile pigments, as is evinced by the icterus generally associated with this infection. In the present case there are, as would be expected, several blood changes from the chemical composition point of view, e.g. such as the *rapid* drop from the normal Hb. level even if compared with anaplasmosis in which erythrorhexis is also marked ; the striking increase in the sugar content and N.P.N. It is felt, however, that a detailed comparison with anaplasmosis would be more justified when a larger number of cases are available and this aspect is therefore not dealt with at present. The normal data have, however, been utilised in drawing up the "normal ranges" as given under Anaplasmosis.

(*a*) EXPERIMENTAL DATA.

CASE I.

B. 3198. *Redwater. Treated and Recovered.*

Temperature Chart I.



History: B. 3198. A two year old heifer in good condition. Inoculated against anthrax and blackquarter in November, 1930, the anthrax immunity being tested 12 months later. During May–June, 1932, it passed through a severe *Anaplasmosis* reaction (*vide*). On 28/6/32 it received subcutaneously 75 c.c. blood from B. 3735. The parasites (*P. bigeminum*) were first noticed 8/7/32, coinciding with the onset of the temperature reaction. Occasional *A. marginale* reappeared, but the *P. bigeminum* were very numerous and the symptoms, including severe haemoglobinuria, so pronounced that from a practical point of view the presence of the *A. marginale* can be overlooked. This patient received chemotherapeutic treatment (10/7/32), the temperature dropping as a result from 105·6–97·8 per cent. within 24 hours. Prior to the injection the animal was down, showed salivation, icterus, anaemia and dyspnoea, and from a prognostic point of view unlikely to recover.

TABLE 1.

B. 3198. *Redwater* (P. bigeminum). *Treated* (*Recovered*).

Date.................. *Time*..................		1/7/32. —	8/7/32. —	11/7/32. —	12/7/32. —	14/7/32. —	22/7/32. —
Temp. Reaction..........		P.I.N.	R.	R.	R.	N.	N.
Haemoglobin gm. %.....		10·35	8·88	3·58	3·42	5·18	9·60
Sugar mgm. %........	L	60·24	66·67	83·33	158·72	76·34	60·61
	U	53·76	59·52	77·52	147·12	68·03	52·63
Total N. gm. %.........		2·500	2·220	1·548	1·660	1·562	2·444
N.P.N. mgm. %....	L	13·26	12·50	29·42	41·84	19·86	16·72
	U	10·38	9·37	27·21	38·48	16·58	11·00
Coag. N. gm. N %..	L	2·487	2·207	1·519	1·618	1·542	2·426
	U	2·490	2·211	1·521	1·622	1·545	2·433
Urea mgm. N %...	L	3·35	4·00	12·42	26·60	9·75	3·84
„ U %...		7·14	8·40	26·04	55·86	20·58	8·00
mgm. N %...	U	3·00	3·65	12·07	26·58	9·31	4·00
„ U %...		6·30	7·77	25·41	55·86	19·53	8·40
Total Creatinine							
mgm. N %...	L	2·04	2·01	3·48	2·86	2·35	2·27
„ TC %..		5·50	5·40	9·40	7·72	6·36	6·16
mgm. N %...	U	1·44	1·75	3·48	2·50	2·01	1·86
„ TC %..		3·86	4·70	9·10	6·74	5·40	5·02
Uric acid							
mgm. N %...	L	0·33	0·33	0·24	0·37	0·33	0·35
„ UA %..		0·99	1·00	0·73	1·12	1·00	1·04
mgm. N %...	U	0·20	0·21	0·22	0·29	0·24	0·24
„ UA %..		0·59	0·64	0·67	0·87	0·73	0·73
Amino acid	L	5·00	4·40	8·75	8·33	4·30	4·70
mgm. N %.......	U	3·68	2·59	9·09	6·73	3·04	2·54
Rest Nitrogen	L	1·54	0·76	3·73	2·68	2·05	4·56
mgm. N %.......	U	1·06	0·17	1·35	1·38	1·98	1·36
Plasma................		n.u.	icteric	icteric	icteric	slightly icteric	n.u.

Main Features of Analytical Data.

Hb.—This is initially on the low side, the animal not having recovered completely from its previous anaplasmosis infection. With the appearance of the redwater parasites there is a very rapid drop in Hb. level from 8·8–3·6 gm. per cent. within 72 hours. This rapid destruction was reflected in the severe haemoglobinuria which occurred. Regeneration was remarkably rapid, the original level being nearly reached in about ten days' time.

Sugar.—Shows a marked rise during the reaction from 66–158 mgm. per cent. and from 60–147 mgm. per cent. in "laked" and "unlaked" filtrates respectively.

N.P.N.—An increase from ± 12–42 mgm. N per cent. and from ± 10–38 mgm. N per cent. in "laked" and "unlaked" filtrates respectively.

U.N.—Shows similar features—an increase from 4–27 mgm. N per cent. taking place.

T.C.N.—Is highest during the reaction for both filtrates.

A.A.N.—This increases from ± 4·5–8·5 mgm. and from ± 3–9 mgm. for "laked" and "unlaked" filtrates respectively, the acme being reached during the febrile reaction.

T.N.—A drop from 2·5–1·5 gm. per cent. is recorded, coinciding with the decrease in the Hb. level.

R.N. and *U.A.N.*—Nothing unusual.

(b) Summary.

Only one case being available no general deductions as to the changes in the blood resulting from a *P. bigeminum* infection are permissive. Summarising the above case one notices marked increases in nearly all the nitrogenous fractions and in sugar, associated with the period of maximum erythrocyte destruction. The efficacy of the kidneys renders a rapid excretion of all " free " haemoglobin possible, but apparently the excretion of urea is retarded temporarily, leading to an increase in the blood for 2–3 days. The rise in A.A.N. is peculiar, particularly if it be remembered that the animal ceased feeding just during the period in which the A.A.N. was highest. Conceivably the increase is due to the protein decomposition rather than an interference with the absorption from the intestines and deaminisation of amino-acids in the liver. On the other hand the liver has a severe strain placed on it by the secretion of abnormally large amounts of bile pigments as a result of the excessive erythrocyte destruction and deaminosation may thereby be temporarily interferred with. If this is the correct interpretation the increase in urea would find its explanation not in increased formation, but in retention or retarded excretion through the kidneys, possibly due to degenerative changes. More research is necessary before any definite modus vivendi for the various observations can be formulated and substantiated. In the meantime the case is of interest in so far as it indicates some of the changes in composition resulting during a fulminant severe erythrolysis.

IV. ACKNOWLEDGMENTS.

In conclusion I wish to record my appreciation of and indebtedness to my colleagues Messrs. W. O. Neitz and B. S. Parkin for their co-operation in permitting me to bleed their experimental animals, whenever required and for access to their records; also to Mr. W. F. Averre and assistants for performing the bleeding.

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Graf, H., I. Comparative studies on blood, "laked" and "unlaked" blood filtrates of animals in health and disease, with particular reference to methods and technique employed.

Jorden, T. J. and Graf, H., II. A contribution to the determination of urea in animal blood filtrates ("lakes" and "unlaked").

Graf, H., III. Comparative studies on "laked" and "unlaked" blood filtrates of sheep in health and during Heartwater (*Rickettsia ruminantium* infection) and Bluetonge (Catarrhal fever).

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Graf, H., V. (This article).

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UNION OF SOUTH AFRICA

DEPARTMENT OF AGRICULTURE

THE ONDERSTEPOORT JOURNAL OF VETERINARY SCIENCE AND ANIMAL INDUSTRY

VOL. I OCTOBER, 1933 No. 2

PUBLISHED QUARTERLY

Edited by: P. J. DU TOIT, Director

THE GOVERNMENT PRINTER, PRETORIA, SOUTH AFRICA
1933

4669—15/7/33—1,400

DEPARTMENT OF AGRICULTURE,
DIRECTOR OF VETERINARY SERVICES AND ANIMAL INDUSTRY,
ONDERSTEPOORT LABORATORIES,
PRETORIA, SOUTH AFRICA,
OCTOBER, 1933.

List of Reports issued by the Director of the Onderstepoort Laboratories.

Report of the Government Veterinary Bacteriologist of the Transvaal for the year 1903-4.*
Report of the Government Veterinary Bacteriologist of the Transvaal for the year 1904-5.*
Report of the Government Veterinary Bacteriologist of the Transvaal for the year 1905-6.*
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Thirteenth and Fourteenth Reports of the Director of Veterinary Education and Research, Parts I and II, October, 1928.
Fifteenth Report of the Director of Veterinary Services, Parts I and II, October, 1929.
Sixteenth Report of the Director of Veterinary Services and Animal Industry, August, 1930.
Seventeenth Report of the Director of Veterinary Services and Animal Industry, Parts I and II, August, 1931.
Eighteenth Report of the Director of Veterinary Services and Animal Industry, Parts I and II, August, 1932.
Onderstepoort Journal of Veterinary Science and Animal Industry, Vol. I, No. 1, June, 1933.
Onderstepoort Journal of Veterinary Science and Animal Industry, Vol. I, No. 2, October, 1933.

P. J. DU TOIT,
Director of Veterinary Services and Animal Industry.

Now out of print.

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FOREWORD.

SINCE the foundation of the Onderstepoort Veterinary Research Station twenty-five years ago, the results of research work carried out at this Institution have been published in the form of "Annual Reports." This form of publication will, in future, be departed from only as regards title and frequency publication. The intention is that *The Onderstepoort Journal of Veterinary Science and Animal Industry* should form a direct continuation of the "*Reports of the Director of Veterinary Services and Animal Industry,*" and be published quarterly instead of annually. Two quarterly numbers will form one volume, so that two volumes should be completed every year.

The advantages of publishing in short intervals are obvious. Nothing is more disheartening to the research worker than to see the publication of his results delayed for months or years. It is also hoped that the change in the title of the publication will prove to be of value. "Reports" are apt to be regarded as a mere summary of routine duties or a brief indication of the work performed during the year. Such a report which incorporates a summary of the activities of the Division of Veterinary Services and Animal Industry is actually published annually by the Department of Agriculture in *Farming in South Africa.* But the "*Reports of the Director of Veterinary Services and Animal Industry*" were strictly scientific volumes containing original articles written by the research workers themselves. It is felt that the term "*Journal*" will indicate the nature of this publication better than the term "Report."

Number 1 of this Journal appeared in July, 1933.

Finally, the hope is expressed that the new Journal will find a worthy place on the library shelves of all Institutes interested in biological problems.

P. J. DU TOIT.

Onderstepoort,
October, 1933.

Section I.

Protozoal.

W. O. NEITZ ..... Blood Parasites of Game in Zululand. Further Report 411

Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 1, Number 2, 1933.

Blood Parasites of Game in Zululand. Further Report.

By W. O. NEITZ, B.V.Sc., Veterinary Research Officer, Onderstepoort.

IN a previous * paper a list of blood parasites found in game shot in the Hlabisa and Lower Umfolozi magisterial districts of Zululand were placed on record. This work has been continued and the results of the examination of blood, spleen, and gland smears of one hundred and seventy-six game animals from the Lower Umfolozi District are mentioned.

The investigation showed that trypanosomes could be demonstrated only in three animals, namely two bushbuck and one kudu. The trypanosomes found in the bushbuck were identified as *T. vivax*. Some of the trypanosomes seen in the kudu were *T. vivax* and the others were distorted, making it impossible to be identified.

Further observations were made in connection with members of the Family Babesidae and Theileridae in various animals.

For convenience the tabulated results of the examination of the game smears from the Lower Umfolozi District are sub-divided into (i) Empangeni, (ii) Umfolozi River. A plus sign means that parasites were present, a minus sign that no parasites were found and a circle that the smear was badly prepared.

Table I.—List of wild animals from Empangeni showing parasites.

Table II.—List of wild animals from Umfolozi River showing parasites.

---

* Blood Parasites of Game in Zululand. *17th Report D.V.S. and A.I.*, Union of S.A., 1931.

TABLE I.—EMPANGENI.

Date.	Species of Wild Animal.	Smear Number.	Microscopical Examination.									Remarks.
			Small Piroplasma.			Trypanosoma.			Microfilaria.			
			Blood.	Spleen.	Gland.	Blood.	Spleen.	Gland.	Blood.	Spleen.	Gland.	
6.12.29	Bushbuck..	519	+	+	+	—	—	—	—	—	—	
6.12.29	„ ..	545	+	—	—	—	—	—	—	—	—	
6.12.29	„ ..	546	—	+	—	—	—	—	—	—	—	
6.12.29	„ ..	551	+	—	—	—	—	—	—	—	—	
23.12.29	„ ..	586	+	+	+	—	—	—	—	—	—	
23.12.29	„ ..	601	—	—	—	+	+	+	+	—	—	*T. vivax.*
23.12.29	„ ..	624	—	—	—	+	○	○	—	—	—	*T. rivax.*
6.12.29	Duiker....	518	+	+	+	—	—	—	—	—	—	
6.12.29	„	532	—	+	—	—	—	—	—	—	—	
6.12.29	„	534	—	+	—	—	—	—	—	—	—	
6.12.29	„	564	+	—	—	—	—	—	—	—	—	
23.12.29	„	591	+	+	—	—	—	—	—	—	—	
6.12.29	Waterbuck	526	+	+	+	—	—	—	—	—	—	
6.12.29	„ ..	553	—	+	—	—	—	—	—	—	—	
6.12.29	„ ..	583	+	+	+	—	—	—	—	—	—	
25.12.29	„ ..	595	—	—	—	—	—	—	—	+	—	
23.12.29	Kudu.....	602	+	—	—	—	—	—	—	—	—	
23.12.29	„	609	+	+	—	—	—	+	—	—	—	*T. vivax.* Possibly other trypanosomes.
6.12.29	Warthog...	550	—	—	—	—	—	—	—	+	—	
6.12.29	„ ..	558	—	—	—	—	—	—	—	+	—	
6.12.29	„ ..	582	+	—	—	—	—	—	—	—	—	
23.12.29	„ ..	584	—	—	—	—	—	—	—	+	—	
23.12.29	„ ..	598	—	—	—	—	—	—	+	—	—	

TABLE II.—UMFOLOZI RIVER.

Date.	Species of Wild Animal.	Smear Number.	Microscopical Examination.									Remarks.
			Small Piroplasma.			Trypanosoma.			Microfilaria.			
			Blood.	Spleen.	Gland.	Blood.	Spleen.	Gland.	Blood.	Spleen.	Gland.	
18.11.29	Zebra.....	499	+	+	—	—	—	—	—	—	—	
29.10.29	Bushbuck..	446	+	—	—	—	—	—	—	—	—	
18.11.29	,, ..	469	+	+	O	—	—	O	—	—	O	
18.11.29	,, ..	486	O	+	+	—	—	—	—	—	—	Koch's bodies.
18.11.29	Duiker....	458	+	+	+		—	—	—		—	
18.11.29	,, ...	447	+	—	—	—	—	—	—	—	—	
18.11.29	,, ...	478	+	+	+	—	—	—	—	—	—	
18.11.29	,, ...	494	O	+	O				—	—	—	
18.11.29	,, ...	501	+	+	+	—	—					
18.11.29	,, ...	506	+		—	—	—	—	—	—	—	
	Waterbuck	449	+	+	+	—	—	—	—	—	—	
18.11.29	,,	455	+	+	O		—	—	—	—	—	
18.11.29	,,	471	+	+	+	—	—	—	—	—	—	
18.11.29	,,	472	+	—	—	—		—	—	—	—	
18.11.29	Warthog...	491	—	—		—	—	—	+	—	—	
18.11.29	,, ..	492	—	—	—	—	—	—	—	+	—	
18.11.29	,, ..	497		—	—				+	—	—	

Details regarding the species of animals, total number examined from the Lower Umfolozi District, the number showing small piroplasma, trypanosoma or microfilaria are given in the subjoined Table III.

TABLE III.

Animal.	Total number examined.	Number found infested with small Piroplasma.	Number found infested with Trypanosoma.	Number found infested with Microfilaria.
Zebra................	24	1	—	—
Bushbuck.............	50	8	2	1
Duiker...............	36	11	—	—
Reedbuck.............	1	—	—	—
Kudu.................	5	2	1	—
Warthog..............	34	1	—	7
Steenbuck............	5	—	—	—
Blue Wildebeest......	5	—	—	—
Waterbuck............	13	7	—	1
Buffalo..............	1	—	—	—
Wild Dog.............	2	—	—	—

Particulars concerning the species of animals, total number examined from Empangeni, the number showing either small piroplasma, trypanosoma, or microfilaria are given in the attached Table IV (*a*).

TABLE IV (*a*).

Animal.	Total number examined.	Number found infested with small Piroplasma.	Number found infested with Trypanosoma.	Number found infested with Microfilaria.
Zebra..................	15	- -	—	—
Bushbuck..............	32	5	2	1
Duiker................	22	5	—	—
Kudu.................	5	2	1	-
Warthog...............	22	1	—	4
Steenbuck.............	2	—	—	—
Blue Wildebeest........	2	—	—	—
Waterbuck.............	6	3	—	1

Details regarding the species of animal, total number examined from Umfolozi River, the number showing either small piroplasma, trpyanosoma or microfilaria are given in the following Table IV (*b*).

TABLE IV (*b*).

Animal.	Total number examined.	Number found infested with small Piroplasma.	Number found infested with Trypanosoma.	Number found infested with Microfilaria.
Zebra..................	9	1	—	—
Bushbuck..............	18	3	—	—
Duiker................	14	6	—	—
Reedbuck..............	1	—	—	—
Warthog...............	12	—	—	3
Steenbuck.............	3	—	—	—
Blue Wildebeest........	3	—	—	—
Waterbuck.............	7	4	—	—
Buffalo................	1	—	—	—
Wild Dog..............	2	—	—	—

A list giving common and zoological names for the game animals dealt with is given in Table VIII of the Preliminary Report.

DISCUSSION.

1. *Zebra.*—Parasites resembling *N. equi* were found in the one zebra. Only one parasite per erythrocyte was seen.

2. *Bushbuck.*—Small piroplasms of the *Th. mutans* type could be demonstrated in seven animals. In another animal *Theileria tragelaphi* with Koch's bodies in the lymph gland smear was found. Both gamonts and agamonts lying either free or intracellular could be found in rare numbers. (See Figs. I and II.)

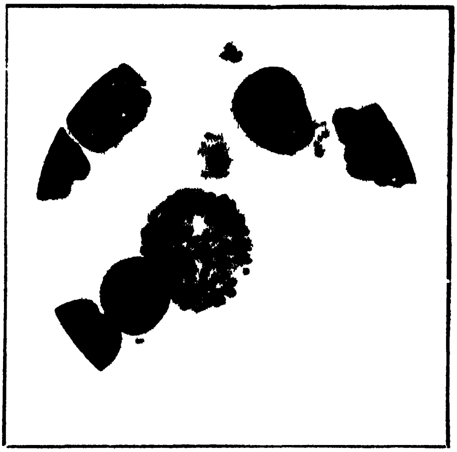

Fig. I. Bushbuck 486 Koch's bodies (agamont) found in gland smear. Magnification 1200 ×.

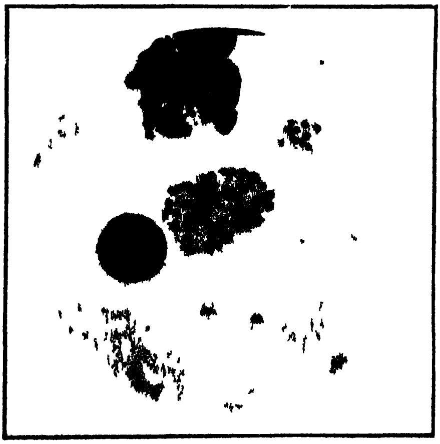

Fig. II. Bushbuck 486. Koch's bodies (gamont) found in gland smear. Magnification 1200 ×.

3. *Duiker.*—Parasites of the *Th. mutans* type were found in eleven animals. In one smear ovoid forms showing division into four producing the cross arrangements were seen, indicating that this is one way in which the parasite multiplies.

4. *Kudu.*—Two animals showed small piroplasms of the *Th. mutans* type. The mode of multiplication is still unknown.

5. *Waterbuck.*—In seven animals small piroplasms of the *Th. mutans* type could be demonstrated.

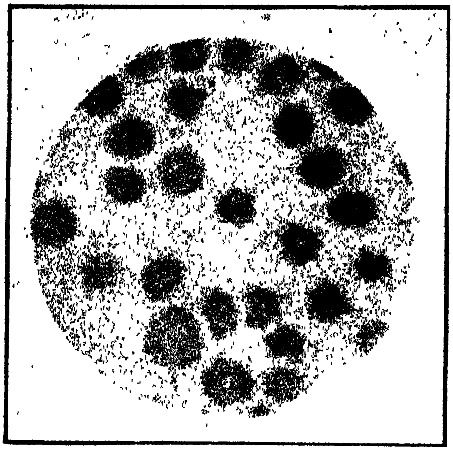

Fig. III. Warthog 582. Four small piroplasms in a single erythrocyte found in blood smear. Magnification 1200 ×.

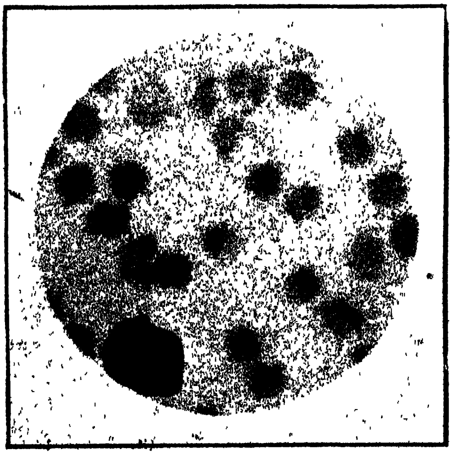

Fig. IV. Warthog 582. Small piroplasms found in Blood smear. Magnification 1200 ×.

6. *Warthog.*—In one warthog small piroplasms of the *Th. mutans* type could be found in the blood smears in very rare numbers. The organisms measured 0·5μ–1·75μ by 0·5μ–1·0μ. The forms seen were ovoid and ringshaped but more than one parasite was found in a single erythrocyte, except in one case, where four parasites were seen giving one the impression of a recent division. No mention of this parasite is made in the literature. (See Figs. III and IV.)

CONCLUSION.

1. *T. vivax* was found in three game animals, viz., two bushbuck and one kudu.

2. Small piroplasms were demonstrated in the zebra, bushbuck, duiker, kudu, waterbuck and warthog. The parasites in the last animal are mentioned for the first time.

3. Microfilaria were found in one bushbuck, one waterbuck and seven warthogs.

LITERATURE.

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Section II.

Mineral Metabolism.

P. J. DU TOIT, A. I. MALAN, AND J. W. GROENEWALD ... Studies in Mineral Metabolism, XXVII. The Effect of Two Different Calcium Phosphorus Ratios upon the Growth of Calves ... 421

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Onderstepoort Journal of Veterinary Science and Animal Industry, Volume I, Number 2, 1933.

Studies in Mineral Metabolism XXVII.

The Effect of Two Different Calcium Phosphorus Ratios upon the Growth of Calves.

By P. J. DU TOIT, B.A., PH.D., DR.MED.VET., D.SC.(AGRIC.), Director of Veterinary Services;
A. I. MALAN, D.Sc., and
J. W. GROENEWALD, M.Sc., Research Officers, Onderstepoort.

EIGHT Red Poll x Friesland cross-bred calves, eight to eleven weeks old, became available and had to be reared under laboratory conditions where observations on food intake and weight increase were part of the daily routine of the station. It was, therefore, decided to divide these calves into two groups receiving the same basal ration, and to vary the calcium phosphorus ratio by the addition of supplements of calcium carbonate and di-sodium phosphate to the basal ration. It was realized that such an experiment could not claim to be a serious attack on the problem of the effect of calcium phosphorus ratios upon growth, but still, data would be provided upon the practical aspect of the effect of a change in the calcium phosphorus ratio upon growth or weight increase, especially in this country where during periods of drought or even winter the phosphorus content of the pasture is low and the ratio of Ca:P definitely different from that which exists during the period of abundant food. Mature pasture containing approximately ·4 per cent. CaO and ·15 per cent. P_2O_5 is common in Bechuanland as well as in other parts of the Union during winter. The ratio of CaO to P_2O_5 in such pasture is about 1 to 0·4 with phosphorus definitely deficient and the effect of such deficiency soon apparent upon the grazing animals. The practical problem, so far, has been the removal of aphosphorosis by giving phosphatic supplements such as calcium phosphate, bone meal, sodium phosphate, etc., without regard to the effect upon the calcium phosphorus ratio or without a conscious attempt to alter the latter. As a matter of fact, the urgency of a solution for the problem of aphosphorosis in this country and the success which has attended attempts to overcome the deficiency by the supply of phosphatic supplements has fostered a certain amount of scepticism in regard to the practical value of calcium phosphorus ratios in cattle farming. It was, therefore, with a view to test the practical significance, i.e. the effect on growth, of a decided change in the Ca:P ratio of the ration of calves that the experiment to be described was undertaken.

It is freely admitted, of course, that the study of calcium phosphorus ratios is fundamental and essential in all work upon calcium and phosphorus metabolism. But it appears that the conflicting results obtained by investigators are due in part to the absence of differentiation between work upon this problem under conditions of a deficiency of either, phosphorus or calcium, or both, and ratio work when both the elements are present in sufficiency. However, the problem of phosphorus and calcium metabolism presents unlimited scope to the investigator and certain aspects have certainly received a very fair share of attention, but information on the interrelation of Ca and P at different levels or concentrations is still meagre according to Bethke, Kick and Wilder (1932).

Without attempting to discuss the available literature it may be said that apparently the concensus of opinion amongst workers upon the problem of calcium phosphorus metabolism under conditions of sufficiency, is that, broadly speaking, a ratio of calcium to phosphorus between the extremes of 1 to ·5 and 1 to 2 may be labelled as a good ratio, while values beyond these limits show detrimental effects upon the animal. Obviously, however, the ratio may be acceptable in the above sense but a deficiency of either calcium or phosphorus may exist with its associated ill-effects upon the animal subject to such conditions. The severity of a deficiency of either phosphorus or calcium may be minimized when working with a most favoured ratio, which usually also lies between the limits mentioned. Marek and Wellman, whose publication Die Rachitis (1932) has been mentioned elsewhere,* do not stress calcium phosphorus ratios, but claim that under all conditions, i.e. sufficiency or deficiency of Ca and P the Erdalkali-alkalizität of a good ration must lie between specific limits. This view is interesting, but requires further work to substantiate it.

DETAILS OF THE EXPERIMENT.

The object of the experiment under discussion was to keep one group of four calves on enough milk to ensure an adequacy of Ca and P intake, and to adjust the calcium phosphorus content of the diet in such a way when supplementary feeding became necessary that the ratio of Ca to P remained the same as that of whole milk, viz., $CaO:P_2O_5:1:1{\cdot}4$ approximately. This ratio is in fair agreement with that usually given, as a glance at the following table will indicate:—

Author.	CaO.	P_2O_5.	$CaO : P_2O_5$.
Richmond (1920)	202·7	293·3	1 : 1·4
Stocking, W. A. (1922)	200·1	243·9	1 : 1·2
Hawk & Bergeim (1927)	235	265	1 : 1·13
Rogers (1928)	202·7	293·3	1 : 1·4
Onderstepoort (several workers)	225·4	327·5	1 : 1·4
Hart, Steenbock *et al.* (1930)	155·0	240	1 : 1·4

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* Studies in Mineral Metabolism. Du Toit, Malan, Groenewald, in a later number of this Journal.

If anything, our values for P_2O_5 are slightly higher than those given by other workers, although perhaps not sufficiently so to warrant serious attention.

The second group of four calves was given, in addition to the milk given to their companion group, calcium carbonate to alter the ratio of $CaO:P_2O_5$ from 1 : 1·4 to 1 : ·33. Here also in the course of the experiment $CaCo_3$ was added to keep the ratio of $CaO:P_2O_5$ constant throughout. Each group received 10 lb. of milk daily for the full period of the experiment which makes a consideration of a possible vitamin D deficiency redundant.

The intake of food of the calves in both groups remained exactly the same as will be evident from a study of Table I:—

TABLE I.

Group A.—Daily Ration per Calf.

Date.	Milk.	Hay.	Maize.	Oats.	$CaCO_3$.	CaO.	P_2O_5.	$CaO:P_2O_5$.
	lb.	gm.	gm.	gm.	gm.			
28.7.31	10	—	—	—	-	10·10	14·7	1 : 1·45
7.8.31	10	100	-	—	—	10·50	15·0	1 : 1·40
17.8.31	10	200	—	—	-	10·9	15·3	1 : 1·4
5.9.31	10	450	-	—	—	12·1	16·0	1 : 1·3
10.9.31	10	450	220	220	2·5	13·6	18·5	1 : 1·4
12.10.31	10	450	450	450	2·0	14·0	20·7	1 : 1·4
5.11.31	10	900	450	450	—	15·6	21·8	1 : 1·4
18.11.31	10	900	900	450	2·0	16·9	23·6	1 : 1·4

TABLE II.

Group B.—Daily Ration per Calf.

Date.	Milk.	Hay.	Maize.	Oats.	$CaCO_3$.	CaO.	P_2O_5.	$CaO:P_2O_5$.
	lb.	gm.	gm.	gm.	gm.			
28.7.31	10	—	—	—	68	46·5	14·7	1 : 0·3
7.8.31	10	100	—	—	68	47·0	15·0	1 : 0·3
17.8.31	10	200	—	—	68	47·4	15·3	1 : 0·3
5.9.31	10	450	—	-	68	49·0	16·0	1 : 0·3
10.9.31	10	450	220	220	80	58·4	18·5	1 : 0·3
12.10.31	10	450	450	450	100	70·0	20·7	1 : 0·3
5.11.31	10	900	450	450	100	71·6	21·8	1 : 0·3
18.11.31	10	900	900	450	100	72·9	23·6	1 : 0·3

The mineral supplement was added in the milk, which was given twice daily to each calf. The rest of the ration was given in a common trough and the calves of both groups given access simultaneously. The calves were kept in a small bare paddock and allowed into a common shed at night. The supplements of hay, maize and oats were given twice daily and the calves weighed at fortnightly intervals. The average fortnightly weights of both groups of calves for the full experimental period of 9 months are represented graphically in Figure I.

A glance at Figure I shows the perfectly normal and regular trend of the curves of the weights of both groups of calves. The calves in both groups gained steadily in weight and remained in good health and condition throughout the trial. At 12 months of age the average weight of the calves in either group was approximately 370 lb., which agrees very well with the weights of the 1932 calf crop of the same cows by the same bull and hand-reared with milk according to the routine method of this Institute.

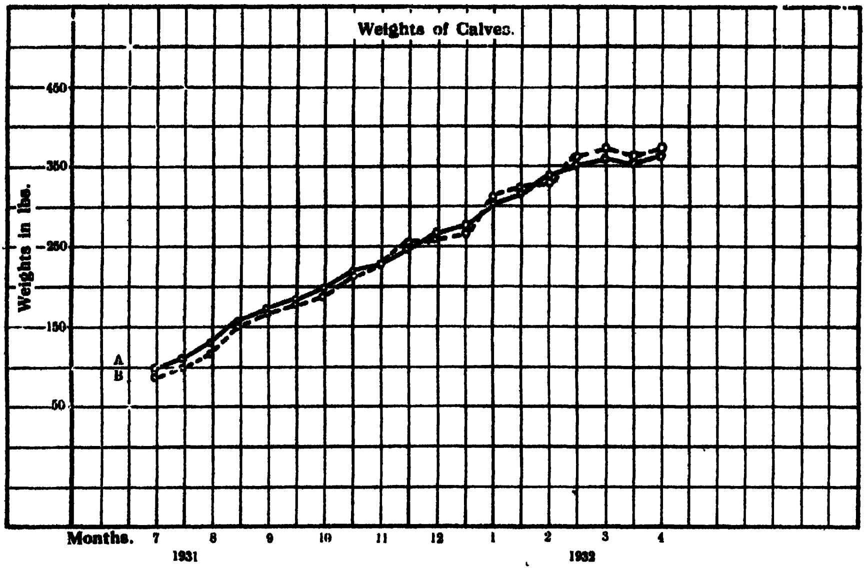

Figure 1.

There is no reason for believing that a ratio of $CaO : P_2O_5$ of 1 : 1·4 produced better growth in the calves under the conditions of the experiment, i.e. probably of calcium and phosphorus sufficiency than a ratio of 1 : 0·3. At all events such a difference, if it existed, was not apparent in the growth curves of the calves during their first year of life, i.e. of high calcium and phosphorus requirements. In conclusion it should be mentioned that as this experiment throws considerable doubt on the practical significance of varying calcium phosphorus ratios in the nutrition of calves, it is being followed up with further work along the same lines.

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Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 1, Number 2, 1933.

The Colorimetric Determination of Sodium in Vegetation.

By J. G. LOUW, M.Sc., Research Officer, Department of Biochemistry, Onderstepoort.

In a recent issue of the Report of the Director of Veterinary Services and Animal Industry, Malan and Van der Lingen (1931) gave details of the Uranyl-zinc-sodium-acetate method for the determining of sodium in blood and vegetation. It was found, however, in the course of the determinations of sodium in grasses in connection with " The Study of the Feeding Value of South African Pastures " (Du Toit et al 1932) that the method did not give concordant results under all conditions. As a matter of fact, it was soon noticed that the values for sodium were inexplicably high on some occasions, and that they could be made to vary at will by altering the potassium content of the aliquot in question and the temperature at which the precipitation of the triple acetate is effected. It was, therefore, decided to investigate the method for the determination of sodium and to develop a reasonably accurate technique suitable for routine procedure as many thousands of determinations had to be carried out in the course of the study.

Since the completion of this work the author noticed that McCance and Shipp (1931) advise the main modification now included in the present method for the determination of sodium. However, it is considered advisable to supplement Malan and Van der Lingen's article with the present one and for that reason full details will be given.

REAGENTS.

1. Precipitating reagent: —

   (*a*) 10 gm. Uranyl-acetate dissolved in 50 c.c. water containing 6 c.c. 30 per cent. acetic acid.

   (*b*) 30 gm. zinc acetate dissolved in 50 c.c. water containing 3 c.c. 30 per cent. acetic acid.

The salts are brought into solution on the water bath, the solutions mixed while still hot, allowed to stand in a cool place for 24 hours, filtered and kept in a sodium-free glass-stoppered bottle.

2. Powdered calcium hydroxide (sodium free).
3. 20 per cent. $K_4Fe(CN)_6$ solution.
4. Absolute alcohol.
5. 96 per cent. alcohol saturated with $(UO_2)_3$ Zn. Na $(CH_3COO)_9$.
6. Standard solution of NaCl containing 0·1 mg. Na/cc.
7. Standard solution of triple acetate.

The triple acetate $(UO_2)_3$ Zn. Na. $(CH_3COO)_9 \cdot 6\ H_2O$, is prepared by mixing 300 c.c. of the above standard solution of NaCl, 900 c.c. absolute alcohol and 600 c.c. of the precipitating reagent. After 30 minutes the precipitate is collected on a filter attached to a suction apparatus, thoroughly washed with 96 per cent. alcohol saturated with the precipitate, and followed by two or three washings with ether. The precipitate is then dried in an electric oven at 103° C. and allowed to cool in a desiccator. 0·6689 gm. of the salt is accurately weighed, dissolved in 50 c.c. 10 per cent. acetic acid in a 100 c.c. volumetric flask and filled to the mark with distilled water. 1 c.c. of this solution is equivalent to ·1 mg. Na. It has been found to keep well for three months.

EXPERIMENTAL.

(*a*) Elimination of Phosphorus.

Malan and Van der Lingen directed that the elimination of phosphorus should be brought about by absolute alcohol saturated with zinc acetate. By testing the supernatant fluid so obtained for phosphate it was, however, observed that alcoholic zinc acetate eliminated phosphorus in an erratic manner. But the phosphate not removed by this means was never found to be precipitated by Uranyl zinc acetate. However, on trying to determine sodium without eliminating phosphate it was found that the precipitate of uranyl zinc phosphate is insoluble in dilute acetic acid and water. Non-elimination of phosphate would mean a saving of time and it was therefore decided to establish to what extent this insoluble phosphate precipitate would interfere with the determination of sodium.

For the first part of the investigation a pure solution of sodium chloride was accurately made up. To this definite amounts of a solution of tricalcium phosphate were added. The procedure for determining Na was that described further on in this article. Colours were compared within 5-10 minutes after development. Results obtained are given in Table I below. Table II shows the effect of eliminating and not eliminating phosphate with powdered $Ca(OH)_2$ on the sodium content of 1 c.c. of a grass extract. The percentage error is reckoned on the value obtained when phosphate has been eliminated. Column 1 gives the amount of phosphorus actually present in the aliquot extract used.

Table I.

	Mgm. Na Taken.	Mgm. P Added.	Vol. made up to.	0.1 mgm. Na std. at 20 Colorimetric Reading.	Mgm. Na Found.	Percentage Recovery.
1	·04	0	25·0 c.c.	24·8	·0404	101·0
2	·04	·1	„	24·8	·0404	101·0
3	·04	·2	„	23·0	·0435	108·7
4	·08	0	50 c.c.	25·0	·08	100
5	·08	·1	„	24·8	·0807	100·9
6	·08	·2	„	24·7	·081	101·25
7	·1	0	„	20·0	·1	100·0
8	·1	·1	„	19·6	·102	102·0
9	·1	·2	„	19·4	·103	103·0
10	·2	0	100 c.c.	20·0	·2	100·0
11	·2	·2	„	20·0	·2	100·0
12	·2	·3	„	20·0	·2	100·0
13	·2	·4	„	20·6*	·194	97·0
14	·4	·4	„	10·1	·396	99·0
15	·8	0	„	5·0	·8	100
16	·8	·4	„	5·0	·8	100

* Compared three minutes after development of colour.

Table II.

Extract No.	Mgm. P in Aliquot Used.	Mgm. Na Found in Aliquot without Removing P.	Mgm. Na Found in Aliquot after Eliminating P.	Percentage Error.
1............	·035	·033	·027	+ 22·2
2............	·012	·035	·029	+ 20·7
3............	·20	·02	·01	+ 100·0
4............	·14	·015	·011	+ 36·3
5............	·16	·015	·01	+ 50·0
6............	·15	·014	·01	+ 40·0
7............	·12	·015	·011	+ 36·3
8............	·17	·015	·01	+ 50·0
9............	·14	·042	·032	+ 31·2
10............	·18	·145	·141	+ 2·8
11............	·28	·235	·231	+ 1·7
12............	·33	·43	·41	+ 4·8
13............	·73	·586	·570	+ 2·8
14............	·05	·12	·119	+ ·84
15............	·05	·10	·10	0
16............	·07	·153	·148	+ 3·4
17............	·06	·126	·125	+ 0·8
18............	·09	·204	·204	0
19............	·09	·114	·114	0
20............	·21	·17	·167	+ 1·8

A glance at Tables I and II brings out the fact that if the phosphorus content of the specimen to be tested for sodium is not unduly high reasonably accurate values are obtained between the limits ·04—·8 mgm. Na. The interference of phosphorus is limited to a slight turbidity in the developed colour. In Table I the greatest error occurs in No. 3, where ·2 mg. P was present in an aliquot containing ·04 mg. Na and the volume was made up to 25 c.c. The phosphate precipitate was dispersed through the whole solution in a finely divided condition giving the developed colour a distinct milky appearance. If this colour is compared 2 or 3 minutes after development a low value is obtained against a standard of the pure sodium triple acetate, 10 minutes after the colour intensity has appreciably increased, so much so that a result above the theoretical amount of Na is obtained. With the same amounts of phosphorus present but higher quantities of Na and consequently larger volumes in which colours are developed this effect becomes negligible, the error introduced being within the limits usually encountered in colorimetric work.

With all sodium values below ·04 mgm. where the volume is made up to only 10 c.c. the interference of phosphorus becomes more marked. Apart from the fact that even when phosphorus has been eliminated values differing by as much as 30 per cent. for the same test sample have been obtained, the elimination here is advisable especially when the phosphorus content of the sample to be analyzed is high.

The use of $Ca(OH)_2$ in powder form has been found a rapid and reliable method for eliminating phosphorus. For the purpose of routine work on grasses it will seldom be found necessary to resort to elimination. In the present method the potassium content of a grass determines the aliquot allowable in the determination of sodium. In the case of green grasses with high potassium content aliquots as low as 0·2 c.c. have to be used. This at the same time brings the phosphorus content of the sample to be tested for sodium to such a low level that interference from this source will be serious only in those samples having a low sodium content, when, in addition, the volume in which the colour has to be developed is only 10 c.c. However, when the sodium level drops to such a low value that phosphorus interferes under the conditions described an accurate determination is hardly necessary for the difference in intake of sodium by an animal on pasture containing ·01 or ·02 per cent. is negligible as either value gives an exceedingly low intake compared with the requirements of the animal.

(*b*) INTERFERENCE OF POTASSIUM.

Especially on cold winter days a coarse yellow crystalline precipitate as distinct from the fine precipitate obtained from sodium alone was deposited on the sides of the precipitating tube. This precipitate was found to be readily soluble in dilute acetic acid and water. Results could seldom be reproduced, especially when duplicates were tried on hotter days or when a different aliquot was used in the determination. The following table is given to illustrate the point:—

Table III.

	PRECIPITATING TEMPERATURE: 26° C.			PRECIPITATING TEMP.: 20° C.
	Mgm. Na per c.c. extract when 0 2 c.c. is used.	Mgm. Na per c.c. extract when 0·5 c.c. is used.	Mgm. Na per c.c. extract when 1 c.c. is used.	Mgm. Na per c.c. extract when 1 c.c. is used.
1	·047	·133	·69	1·08
2	·029	·258	·66	1·05
3	·029	·053	·33	·89
4	·032	·037	·31	·67
5	024	·046	·33	·68
6	32	·42	·49	1·11

Sjollema and Dienske (1931) stressed the interference of potassium when the ratio of Na:K exceeds 1:20 due to the co-precipitation of a Pot. uranyl salt. To overcome this difficulty the authors resorted to partial elimination of potassium by means of tartaric acid. Apart from the fact that under certain conditions of temperature the residual potassium may still interfere with an accurate determination, the procedure is much too laborious to be of practical value in our work.

Tests were undertaken by the author in order to satisfy himself as to the reliability of Sjollema and Dienske's conclusion on the one hand, and to ascertain the reason for inconsistent results obtained in analysing grass extracts on the other. Numerous tests with known solutions of NaCl and KCl were undertaken, the concentrations and ratios of Na:K being varied within wide limits. K was found to interfere but this interference was seldom found to be the same for any one concentration of K. Further, it was established that this interference was not dependent upon the ratio of Na:K, but upon the absolute concentration of K in the test sample.

The inconsistency of interference was established to be due to difference in temperature in the precipitating medium. The following table illustrates this point while giving at the same time an indication as to the concentration of K at which precipitation commences. 2 c.c. precipitating reagent were added to a mixture of 3 c.c. absolute alcohol and 1 c.c. distilled water containing varying amounts of K as KCl. The precipitates obtained were washed with 96 per cent. alcohol saturated with sodium zinc uranyl acetate, dissolved in 0·5 c.c. 10 per cent. acetic acid and made up to definite volumes with water. Colours developed and matched against a 0·1 mg. Na standard. Results given as mgm. sodium:—

Table IV.

	Mgm. K in 1 c.c. Water.	Precipitation at 26° C. Mgm. Na Found.	Precipitation at 10° C. Mgm. Na Found.
1	0·4	—	—
2	0·5	—	—
3	0·6	—	Trace.*
4	0·7	—	Trace.
5	0·8	—	Trace.
6	0·9	Trace	·009
7	1·0	·007	·11
8	2·0	·23	40
9	3·0	·33	·66
10	4·0	·53	1·0

* Traces increase from Nos. 3 to 5.

According to this table potassium will begin to be precipitated in the cold at a concentration of only 0·6 mg. in the test sample whereas at the higher temperature interference commences at 0·9 mgm. However, in the presence of sodium, especially at high concentrations, one will expect less potassium to be precipitated, probably due to preferential precipitation of sodium and more of the potassium precipitate dissolving in the diluted reagent. The expectation was borne out by experiment as is clear from the following table of results:—

Table V.

PRECIPITATION AT 20° C.

	Mgm. Na Present.	Mgm. K Present.	Mgm. Na Found.	Percentage Error.
1	·05	3·0	·125	+ 150·0
2	·10	3·0	·164	+ 64·0
3	·20	3·0	·285	+ 52·5
4	·40	3·0	·50	+ 25·0
5	·60	0·6	·60	0
6	·60	3·0	·78	+ 30·0
7	·35	·50	·348	— 0·57
8	·35	2·5	·50	+ 42·8
9	1·00	·40	·98	— 2·0
10	1·00	1·00	1·06	+ 6·0
11	·80	·30	·79	— 1·25
12	·80	·80	·84	+ 5·0

(c) EFFECT OF TIME AND TEMPERATURE ON THE PRECIPITATION OF SODIUM.

Since the solubility of pot. zinc uranyl acetate in the precipitating medium varies greatly with temperature, it was thought advisable to ascertain the behaviour of sodium zinc uranyl acetate towards temperature and, incidentally, whether the usual 30 minutes was sufficient for complete precipitation of the sodium.

A solution of NaCl containing ·1 mg. Na per c.c. was used to test these points. One series of 1 c.c. test samples were taken through all the stages of the method, allowing precipitation to proceed at the laboratory temperature (26° C) for one hour, while a second series was similarly treated except that the tubes were placed during precipitation in a basin containing iced water registering 2° C. In a third series the sodium was precipitated at room temperature (26° C) for 30 minutes. All the precipitates were dissolved in 50 c.c. water containing 0·5 c.c. 10 per cent. acetic acid, the colours developed and matched against a standard colour from 1 c.c. of a triple acetate solution made up to 50 c.c. with water. 1 c.c. of this triple acetate solution is equivalent to 0·1 mg. Na. The standard was placed at 20 m.m. Colorimetric readings are given in Table VI.

Table VI.

First Series Room Temperature. One Hour.	Second Series 2° C. One Hour.	Third Series Room Temperature. 30 Minutes.
20·2	20·0	20·2
20·3	19·9	20·8
20·3	20·0	20·6
19·9	19·9	20·0
Averages: 20·17	19·95	20·4

The second series is closest to the theoretical reading, viz. 20·0, while the third series shows the highest error. If it is remembered, however, that the present method is intended only for routine analysis of vegetation, there is no reason to prolong the time of precipitation to 1 hour, nor for allowing it to take place at the lower temperature which has in addition the drawback of allowing potassium to interfere at lower concentrations. Tests were also made which show that the same negligible error is made when amounts of Na up to 0·9 mgm. is present in the test sample, allowing precipitation to proceed for 30 minutes at room temperature.

(d) WASHING THE PRECIPITATE.

When all the foregoing points are taken into consideration in the determination of sodium inconsistent results will still be obtained if the precipitate is washed three times with absolute alcohol as described by Malan and Van der Lingen. This difficulty was overcome by following the procedure of Salit (1931) except that 96 per cent. alcohol saturated with the triple acetate is used instead of his more expensive concentrated acetic acid. The washing mixture is prepared by adding about 2 grams of uranyl-zinc-sodium-acetate to 3 litres of 96 per cent. alcohol, shaking the flask vigorously several times and then putting it away in the ice chest (10° C) for at least a day before being filtered for use.

(*e*) DETAILED DESCRIPTION OF METHOD.

Sodium and potassium are determined in the same extract of vegetation. Full details for the preparation of the extract are given by Malan and Van der Lingen (1931). Briefly, the HCl extract of the ash of approximately 10 gm. dry grass is made up to 100 c.c. with distilled water. The sodium estimations follow after the potassium determinations so that the amount of K present in the aliquot for sodium determination will be known. The elimination of phosphorus will be included for the sake of completeness, but in the majority of analyses this step was omitted in the routine procedure employed for the determination of sodium without more than a negligible experimental error.

Into a conical centrifuge tube of 10 c.c. capacity is pipetted about 5 c.c. of the extract and about 0·2 gm. $Ca(OH)_2$ added by means of a small round spoon, which when full contains roughly this amount. Close the tube with a rubber stopper, shake vigorously, and after allowing it to stand for five minutes centrifuge for three minutes. Transfer by means of a pipette graduated to 0·02 c.c. a definite volume of the supernatant, not more than 1 c.c. and containing not more than 0·8 mgm. K, to another conical centrifuge tube containing 3 c.c. absolute alcohol. If less than 1 c.c. of the dephosphated extract has been measured out the difference is made up with doubly distilled water so that the total added to the 3 c.c. absolute alcohol is 1 c.c. Now add 2 c.c. of the precipitating reagent, close the tube with a clean rubber stopper and invert several times. Remove the stopper carefully, wiping the surface that has been in contact with the liquid on the edge of the tube. 1 c.c. Standard solution is treated similarly. After 30 minutes centrifuge for 5 minutes at 2,000 r.p.m. Carefully decant the supernatant fluid, invert the tubes on a piece of filter paper and allow to drain for 10 minutes. With a piece of cloth moistened at one end with alcohol the mouths of the tubes are then wiped clean, and 5 c.c. of the cold washing mixture allowed to run slowly from all round the edge into the tube. Now stir up the precipitate, dispersing it evenly through the whole liquid. Centrifuge for 5 minutes, decant supernatant fluid and allow to drain for at least 15 minutes, after which the mouths of the tubes are again wiped with a dry cloth. The precipitate is dissolved in 0·5 c.c. 10 per cent. acetic acid and a few c.c. of water. The solution is then quantitatively transferred to a glass tube of even bore, bearing a 25 c.c., 50 c.c. and 100 c.c. mark. If the apparent bulk of the precipitate from the test sample is twice that from the 0·1 mg. Na standard its solution is made up to 100 c.c., and if half as much to 25 c.c. The standard is made up to 50 c.c.

A standard is also made up from the standard triple acetate solution by diluting 1 c.c. to 50 c.c. 0·25 c.c. potassium ferrocyanide solution is then added for every 25 c.c. solution. The tubes are inverted to mix and the colours compared in a Duboscq colorimeter after 4 minutes using the triple acetate solution as standard at 20 m.m. The 0·1 mg. Na standard should read at 20·0 mm. When a series of determinations, say 30, are done at the same time, it was found best, especially when phosphorus has not been eliminated, to develop colours in only 10 samples at a time so that the comparisons thereof are finished about 15 minutes after the colours have been developed.

The majority of grasses are very low in sodium content so that even if the volume has been made up to 25 c.c. the readings obtained are much beyond the range of proportionality for accurate results. In these cases 0·5 c.c. 10 per cent. acetic acid and 9·5 c.c. water are accurately pipetted into the tube containing the dry precipitate. After the whole precipitate has gone into solution 0·1 c.c. of the pot. ferrocyanide solution is added, the tube is inverted several times and the colour compared with the standard as before.

The author uses the precipitate obtained from 1 c.c. standard NaCl solution for gauging the bulk of the unknown precipitate and as a check on the standard made up from the triple acetate solution. When small aliquots, e.g. 0·2 c.c. of a grass extract, are used for a determination, and if at the same time the precipitate is so small that the colour volume has to be made up to 10 c.c., a serious error is introduced into the calculations by traces of sodium present as impurity in the reagents used. For this reason it is advisable always to run a blank with a series of determinations and to make, if necessary, corrections when calculating final results.

SUMMARY.

(1) A revision of the method by Malan and Van der Lingen for the colorimetric determination of sodium in vegetation is described in detail.

(2) Evidence is presented to show that:—

(*a*) ·04–0·1 mgm. Na can be determined with reasonable accuracy in the presence of 0·1 mgm. P; when 0·1–0·2 mgm. Na is to be determined 0·2 mgm. P, and when 0·2–0·8 mgm. Na, up to 0·4 mgm. P may be present.

(*b*) The interference of K is dependent upon absolute concentration and temperature; working at ordinary laboratory temperature (± 25° C.) not more than 0·8 mgm. K should be present in the aliquot for a determination.

(*c*) At 25° C. precipitation is complete within experimental error in 30 minutes.

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APPENDIX.

Method for calculating true percentage Na_2O in vegetation when a small precipitate, due to impurities in the reagents, in the blank necessitates a correction.

Example :—

(1) Data :

(i) Weight grass in 100 c.c. extract	x gms.
(ii) Volume extract used for determination	t c.c.
(iii) Colour volume for blank	10 c.c.
(iv) Colour volume for test sample	y c.c.
(v) 0·135 mg. Na_2O standard in 50 c.c. at—	
(*a*) A m.m., test sample reading	a
(*b*) B m.m., blank reading	*b*

(2) Correct value :

$$= \left\{ \frac{A}{a} \times \cdot 135 \times \frac{y}{50} \times \frac{1}{t} \times 100 \times \frac{100}{x} \times \frac{1}{1{,}000} \right\} -$$

$$\left\{ \frac{B}{b} \times \cdot 135 \times \frac{10}{50} \times \frac{1}{t} \times 100 \times \frac{100}{x} \times \frac{1}{1{,}000} \right\}$$

$$= \left\{ \frac{\cdot 135}{50} \times \frac{1}{t} \times 100 \times \frac{100}{x} \times \frac{1}{1{,}000} \right\} \left\{ \frac{Ay}{a} - \frac{10\,B}{b} \right\}$$

$$= \frac{\cdot 027}{tx} \left\{ \frac{Ay}{a} - \frac{10\,B}{b} \right\} \%\ Na_2O.$$

Section III.

Plant Studies and Poisonous Plants.

C. RIMINGTON AND D. G. STEYN *Psilocaulon absimile* N.E.Br. as a Stock Poison. I. Determination of Oxalic, Malic, Tartaric Acids, etc. 439

Onderstepoort Journal of Veterinary Science and Animal Industry, Volume I, Number 2, 1933.

Psilocaulon absimile N.E.Br. as a Stock Poison.

I. Determination of Oxalic, Malic, Tartaric Acids, etc.

By CLAUDE RIMINGTON, M.A., PH.D., B.SC., A.I.C., Research Fellow under the Empire Marketing Board, and

D. G. STEYN, B.Sc., Dr.Med.Vet., Research Officer, Onderstepoort.

Registered Number: Onderstepoort Spec. No. 626; 13/5/32. Nat. Herb. No. 11546.

Common Names: Asbos; loogbos.

Origin: Prieska.

State and Stage of Development: Fresh and in post-seeding stage.

ONE of the authors (D. G. S) investigated mortality in Angora goats in the Willowmore District and strongly suspected a *Psilocaulon* sp. (Nat. Herb. No. 8819) of being the cause. The interesting observation was made that 50 grams of this *Psilocaulon* sp. collected on the farm, where the mortality occurred, caused death in rabbits, whilst 120 grams of specimens of this plant collected on the above farm and planted at Onderstepoort produced no ill-effects in rabbits. It was suggested that the plant grown at Onderstepoort had decreased in toxicity as there is a vast difference in the nature of the soil and climatic conditions in the Willowmore District and at Onderstepoort (Steyn, 1931). One hundred grams of fresh *Psilocaulon absimile* N.E. Br. administered to rabbits caused laboured respiration, tympanites, pronounced salivation, accelerated heart beat, symptoms of paralysis within an hour and death with symptoms of asphyxia within three hours after dosing. Fifty grams of this plant in the fresh state given on each of two consecutive days to rabbits produced no ill-effects.

Drenching experiments proved that the sun-dried plant had not decreased in toxicity.

The following lesions were present at autopsy: hyperaemia and oedema of the lungs; dilatation of the heart ventricles; marked hyperaemia and swelling of and haemorrhages in the gastric mucosa.

The results of chemical investigations revealed the fact that at least two toxic principles are present in the plant, one of which forms an insoluble lead salt and is probably an organic acid, whilst the second toxic factor passes through into the filtrate from the lead acetate precipitation, and is of an entirely different nature both chemically and pharmacologically. In the sample of *Psilocaulon* examined, both principles were present in such quantity as to cause death when administered separately in their respective proportions. No synergistic effect could be observed, but death in the field would appear to be due to the toxic principle, the isolation and chemical examination of which is described in the second communication of this series rather than to the organic acids present in the plant. The present paper deals only with the investigation of the organic acid fraction.

Preliminary experiments having shown that the plant contained a considerable quantity of oxalic acid together with lesser quantities of malic, tartaric acid, etc., a portion of the material was worked up in the following manner and quantitative determinations made of these constituents.

Since the fresh material dries only very slowly in the air, it was introduced into a large oven, the temperature of which was maintained at about 95°, and dried until practically constant in weight. The desiccated material was then ground in a coffee mill affording a light brown powder which was employed for the subsequent determination.

MOISTURE CONTENT.

A 100 gm. sample of the fresh, green plant material upon drying until absolutely constant in weight, lost moisture corresponding to 67·75 per cent.

1 gm. portions of the larger bulk of ground material on drying to constant weight at 105° lost, on an average, moisture corresponding to 6·92 per cent. Determinations made upon the powder were in all cases corrected by this amount to relate them to percentages on the dry weight basis.

FREE ACIDITY OF THE AQUEOUS EXTRACT.

Although as mentioned later in the discussion, it is very doubtful whether the organic acids ever occur in the plant in the free state, the fact of their being polybasic and relatively strong acids allows of their occurrence as partially neutralized acid salts. Oxalic acid, for example, is found frequently in the form of potassium hydrogen oxalate. Watery extracts of the fresh plant were markedly acid to litmus and the plant itself on ingestion evidently exerts an irritating action upon the gastric mucosa, hence the fairly high titratable acidity which was found was not unexpected.

A 1 gram portion of the powdered material was extracted at the temperature of the boiling water bath with several successive portions of distilled water, the combined extracts (about 100 c.c.) were filtered and titrated by deci-normal sodium hydroxide until just alkaline to phenolphthalein.

Vol. of N/10 alkali required = 5·15 c.c. per gm. dry material.

ASH LEFT ON IGNITION.

1 gm. portions were incinerated in weighed platinum basins until no further loss in weight occurred. The results were as follows:—

No. 1 Wt. residue left 0·2288 g. = 24·58 per cent. on dry wt.

No. 2 Wt. residue left 0·2292 g. = 24·62 per cent. on dry wt.
Mean result 24·60 per cent.

ALKALINITY, TOTAL, SOLUBLE AND INSOLUBLE OF THE ASH, ALSO ACID-INSOLUBLE RESIDUE (SILICA, ETC.).

Ash No. 1 was used for the determination of total alkalinity, whilst Ash No. 2 was treated in such a way that the water-soluble and acid-soluble fractions were titrated separately.

Ash No. 1 was moistened with water, 50 c.c. of deci-normal sulphuric acid added and the mixture warmed upon the water bath for some minutes, methyl orange was then added as indicator and the excess of acid titrated by decinormal sodium hydroxide.

In the second case, ash No. 2 was thoroughly extracted with hot water, added in successive small quantities, each portion being decanted through an ashless filter paper into a beaker where all was collected, methyl orange added and the liquid then titrated with decinormal sulphuric acid. The extractions were then repeated with small quantities of hot, diluted sulphuric acid, exactly 15 c.c. of a decinormal solution being added, and filtrate and washings titrated to methyl orange with standard alkali. Upon the completion of these operations, the neutralized solution from ash No. 1 was filtered and the two filter papers with their contents separately ignited and the weight of the acid-insoluble residue of sand, silica, etc., thus obtained. The results are collected below:—

No. 1 ash required 37·34 c.c. N/10 alkali = 40·12 c.c./gm. on dry wt.

No. 2 ash water-soluble fraction required 28·4 c.c. N/10 alkali = 30·51 c.c./gm. on dry wt.
acid-soluble fraction neutralized 9·6 c.c. N/10 alkali = 10·31 c.c./gm. on dry wt.
Total = 40·82 c.c./gm. on dry wt.

Wt. of acid-insoluble residues correspond to ... ... 1·02 per cent. on dry wt.
1·01 per cent. on dry wt.
Mean 1·02 per cent. on dry wt.

DETERMINATION OF CALCIUM AND POTASSIUM IN THE ASH.

Qualitative examination of the ash showed the presence of Ca, K, Na in considerable quantities. Iron was present only in traces. The calcium content was determined by dissolving the ash derived from 1 gm. of the plant powder in warm dilute hydrochloric acid, filtering, adding ammonia until alkaline, again acidifying with acetic acid and adding a solution of ammonium oxalate. The mixture was kept on the water bath for some hours and then allowed to cool. The precipitate of calcium oxalate was filtered off, washed, dissolved in warm dilute sulphuric acid and titrated by potassium permanganate in the usual way.

Potassium was determined in the ash from a separate quantity (6 gm.) of the plant.* There was 5·2 per cent. calculated as K_2O.

Vol. of N/100 $KMnO_4$ required = 66·8 c.c.
= 0·01339 gm. Ca.

This represents 1·34 per cent. of Ca in the plant or, when expressed as CaO, 8·19 per cent. of the ash.

The high alkalinity of the ash of *Psilocaulon absimile* is in accordance with the fact that the plant is relatively rich in salts of organic acids. On ignition, the metals are left as carbonates or oxides.

In the districts of South Africa where it grows abundantly, the plant is used by the rural population for the manufacture of soap. By burning and extracting the residue with water a lye is obtained which is boiled with the oil or fat as in the familiar technical process. Locally the plant is known as "loog-as" signifying "lye-ash".

Psilocaulon is closely related to the genus *Mesembryanthemum* comprising plants many of which have a high content of organic acids. Burtt-Davy (1912) describes the use of *Mesembryanthemum mahoni*, N.E. Br. by the natives as a fermenting agent in the preparation of an intoxicating liquor named "khadi". It is said, however, that the root (the portion used) contains a poisonous principle which in time proves injurious to the khadi drinker. In the Bulletin of the Imperial Institute (1912) is summarized the report of an investigation of this root powder, in which it is stated that quantities of oxalates amounting to approximately 3 per cent. by weight of free oxalic acid were found. The poisonous effects upon natives are ascribed to this acid. That the fermentative activity of the roots of *M. mahoni* is in reality due to the mycelia of accompanying fungi, has been well established. The interesting observation was made that these fungi also are capable of producing oxalic acid when grown upon sugar solution (Bull. Imp. Inst. 1916). A *Mesembryanthemum* species, probably *bellidiflorum*, is used by the Hottentots for the softening of animal skins, the juice of the plant being worked into the tissues with the aid of a stone. *Mesembryanthemum crystallinum* is referred to by Dragendorff (1898) as a "soda-plant".

DETERMINATION OF OXALIC ACID.

The determination of the various organic acids when present together in plants is a problem of no little difficulty. In many respects the salts of oxalic, malic, tartaric, succinic and citric acids are closely similar. None of the methods so far proposed are capable of giving quantitative sharp separation. For purposes of identification the ester process devised by Franzen and his co-workers (1921-2) is convenient, but the yields are necessarily far from quantitative. As a more general method of procedure, the scheme proposed by Albahary (1912) wherein differences in the properties of the lead salts of the individual acids are exploited, may be recommended, provided great accuracy is not required.

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* For the carrying out of this determination I am indebted to Mr. Holzapfel of this Laboratory.

In the present case, since widely different quantities of the different acids were present, it was found most practicable to concentrate upon the determination of one acid at a time and to adopt or elaborate methods capable of yielding the most reliable figures for this individual. The determination of each acid is, therefore, considered under a separate heading.

Oxalic acid is most readily separated from plant extracts by precipitation as calcium oxalate from a solution slightly acid with acetic acid. Some calcium tartrate may frequently be associated with the oxalate in this precipitate, but by careful reprecipitation, again from acid solution, a separation may be effected. 1 gm. of the finely-ground plant powder was extracted by two successive portions of 100 c.c. of 1 per cent. hydrochloric acid, the flask with its contents being on each occasion immersed in a boiling water bath for one hour. The fluid was decanted from the plant residue, centrifuged and finally filtered. It was then made alkaline by ammonia and the reaction brought once more to the acid side by the addition of a slight excess of acetic acid.

To the boiling liquid a solution of calcium acetate was then added and the mixture kept upon the water bath for about 2 hours. It was then placed in the ice-chest over night. The precipitate was filtered off, washed with water slightly acidified with acetic acid and finally dissolved in hot, dilute sulphuric acid, the volume of this solution of the crude oxalate precipitate being adjusted to 100 c.c. A titration was made upon an aliquot of this solution, using one-hundredth normal potassium permanganate. A further aliquot, or the remainder of the solution, was then subjected to precisely the same procedure. The end-point found when titrating this solution of the reprecipitated oxalate was invariably sharp, whilst some uncertainty attached to that with the solution of the crude precipitate. Any extra deposit separating out when the main filtrate from the calcium oxalate was kept for further periods in the ice-chest, or adhering to the sides of the flask was separately titrated as above.

Results :—

(*a*) Extract from 1 gm. powder (containing 6·92 per cent. of moisture).

Volume of solution of crude oxalate precipitate = 100 c.c.

5 c.c. aliquot required 9·4 c.c. of 0·009656 N $KMnO_4$.

90 c.c. reprecipitated and made up to 100 c.c.

5 c.c. aliquot of this solution required 8·1 c.c. $KMnO_4$ = 9·0 c.c. $KMnO_4$ in 5 c.c. of the original = 8·69 c.c. N/100.

∴ 7·82 gm. oxalic acid $KMnO_4$/100 gm. plant or 8·40 per cent. on dry wt. basis.

Further small deposit required 4·5 c.c. $KMnO_4$ = 4·345 c.c. N/100 $KMnO_4$.

or 0·21 per cent. oxalic acid on dry wt. basis.

Total oxalic acid found = 8·61 per cent.

(*b*) Extract from 1 gm. plant powder.
Volume of solution of crude oxalate precipitate = 100 c.c.
5 c.c. aliquot required 9·1 c.c. of $KMnO_4$ solution.
5 c.c. reprecipitated; precipitate required 9·0 c.c. $KMnO_4$ solution.
Small deposit on sides of flask required 6·4 c.c.
∴ 8·70 per cent. oxalic acid on dry wt. basis.
Mean of above determinations 8·66 per cent.

DETERMINATION OF TARTARIC ACID.

The filtrates from the two precipitations of calcium-oxalate described in (*a*) above, were combined, concentrated, made very slightly alkaline with ammonia and placed in the ice-chest for some days. The small precipitate of calcium salts which formed was filtered off, dissolved in a little dilute acetic acid and, after further concentration, two volumes of a 10 per cent. alcoholic solution of potassium acetate added. The mixture was vigorously stirred at intervals during the day and left in the ice-chest over night. The small precipitate of potassium bitartrate was filtered off, washed with alcohol until free from acid, dissolved in a little hot water and titrated with decinormal sodium hydroxide using phenolphthalein as indicator.

Volume of alkali required = 0·4 c.c. of N/10.
= 0·5 mg. tartaric acid.
∴ 0·064 per cent. in plant on dry wt. basis.

As a check upon this figure a determination was carried out as follows:—1 gm. of the plant powder was extracted with 1 per cent. hydrochloric acid in the usual way and the extract neutralized with ammonia. Lead acetate solution was then added in slight excess and the precipitated lead salts removed by centrifugation, washed with 50 per cent. alcohol, suspended in water and finally decomposed by passing hydrogen sulphide into the hot solution. The filtrate from the lead sulphide was concentrated to about 30 c.c., a few drops of acetic acid added and then 60 c.c. of 10 per cent. alcoholic potassium acetate. The precipitate of potassium hydrogen tartrate was treated as previously described.

Volume of alkali required = 0·45 c.c. of N/10.
= 0·675 mgm. tartaric acid.
∴ 0·072 per cent. in plant on dry wt. basis.
Mean of above determinations 0·068 per cent.

DETERMINATION OF CITRIC ACID.

Citric acid is most conveniently determined by one or other of the methods depending upon its oxidation to acetone. The acetone formed may be determined by iodine titration (Kogan, 1930), conversion into penta-brom acetone (Hartmann & Hillig, 1930) or as the mercury compound. In our experience the method of Bleyer and Schwaibold (1925) is comparatively simple and capable of yielding good results provided no great quantity of tartaric acid is present. The neutralized solution of the acid is made up to 150 c.c. and refluxed for 3 hours with 5 c.c. of the oxidation reagent for every 0·05 gm. citric acid. An excess of reagent causes no error. The precipitated mercury-acetone compound is filtered off whilst the solution is still warm and washed well with water. Concentrated nitric acid is then added (in the present investigation the paper and precipitate were returned to the reaction flask) followed by excess of a concentrated potassium permanganate solution, concentrated ferrous sulphate being then added in excess to remove unused permanganate and after addition of a few drops of iron ammonium alum as indicator the solution is titrated by decinormal ammonium thiocyanate until a permanent reddish brown coloration is produced.

1 c.c. of N/10 NH_4 CNS = 2·69 mgm. citric acid.

The accuracy of the method was tested upon solutions of pure citric acid.

Using the filtrate from the calcium oxalate precipitate obtained when working up an extract from 1 gm. of plant powder, no citric acid could be detected by this method. An extract of a larger quantity was therefore prepared in the following way:—

> 20 gm. plant powder was extracted by three successive portions of 300 c.c. each of 1 per cent. hydrochloric acid. The combined extracts were concentrated upon the water bath to about 100 c.c., a little decolorizing charcoal added and the solution filtered and made up to a volume of 150 c.c. This was then refluxed with 10 c.c. of reagent. The volume of ammonium thiocyanate required to produce a visible end point was one drop (slightly less than 0·1 c.c.). Citric acid is therefore absent.

DETERMINATION OF MALIC ACID.

Of all the commonly occurring plant acids, malic acid presents the greatest difficulty in the way of its quantitative determination. Its separation from the other oxy-acids requires much care and until recently there was no satisfactory way of evaluating the quantities present in such concentrates. Willard and Young (1930) have recently described a very elegant method for the determination of oxalic, malic, citric, tartaric and some other oxy-acids which is applicable to plant analysis once the difficulty of the separation of these individuals has been overcome. Acetic acid does not interfere, neither does succinic acid cause appreciable error under the conditions described. The process is a volumetric one, a solution of ceric sulphate being used as oxidising agent and the excess finally back titrated by means of a ferrous salt.

Since the method promises to gain in favour, a description of the preparation and standardization of the ceric sulphate solution is here briefly given. Commercial ceric oxide, containing relatively large quantities of the other rare earth metals was used. It was found that 24 gm. was sufficient to yield 500 c.c. of a decinormal ceric sulphate solution. This material was warmed in an evaporating basin with 100 c.c. of sulphuric acid solution of S.G. 1·5, small quantities being added at a time and the mixture continuously stirred. As the water evaporated off, the mass assumed first of all a rich red and then a deep yellow colour. This paste was again warmed with

25 c.c. of the acid and the stirring continued at as high a temperature as conveniently possible until no further lightening in the colour of the paste was observable. The whole operation occupied about 1½ hours. Water was then added to make a volume of about 450 c.c. and the solution kept at 75-80° for one hour after which it was cooled, filtered and adjusted to 500 c.c. Such a solution should be about normal in free sulphuric acid. For standardization a decinormal sodium oxalate solution was allowed to run into an aliquot of the ceric sulphate maintained at about 60°. The disappearance of the intense yellow colour of the ceric salt marks the end point, but as a check, or when titrating tinted solutions, a drop may be mixed externally on a spot plate with a few drops of a diphenylamine or diphenylbenzidine solution. A blue colour indicates the presence of free oxidising agent.

Tested upon citric acid, the method proved perfectly trustworthy. 20 c.c. citric acid solution (approx. 0·2 per cent.) was mixed with 75 c.c. of a 0·049 normal ceric sulphate solution (decinormal is to be preferred), 50 c.c. of sulphuric acid, S.G. 1·5 added, and water to 200 c.c. The mixture was boiled under the reflux condenser for 30 minutes, cooled and titrated with a freshly-prepared standardized ferrous sulphate solution (0·098 normal). The volume of ceric sulphate solution reduced was 64·6 c.c. whence (employing the factor 1 c.c. N/10 $Ce(SO_4)_2$ – 001211 gm. citric acid) the original sample contained 0·1964 gm. per 100 c.c. The solution actually contained 0·1962 gm. citric acid per 100 c.c.

The determination of malic acid is carried out on precisely the same lines. Willard and Young find that 1 c.c. N/10 $Ce(SO_4)_2$ 001449 gm. malic acid. A preliminary experiment which afforded a rough indication of the quantity of malic acid present in the plant material was performed as follows: From a 1 per cent. hydrochloric acid extract, the lead salts of the organic acids were prepared by precipitation after neutralizing with ammonia. This lead precipitate was suspended in dilute acetic acid and the mixture kept at a temperature of 70° whilst being stirred for one hour. After centrifuging and washing, the acetic acid solution which contained the lead malate was made up to a volume of 250 c.c. An aliquot of this was treated with 2 volumes of alcohol and the precipitate filtered off, dried and weighed. Reckoned as lead malate it corresponded to slightly over 9 per cent. of malic acid in the plant powder. More accurate determinations were made by means of the ceric sulphate method, the solutions being first purified by taking advantage in the one case of the solubility of calcium malate in hot lime water and in the other the solubility of ammonium malate in 90 per cent. alcohol. The figures obtained by these two methods showed good agreement. A 1 per cent. hydrochloric acid extract of 2 gm. of the plant powder was precipitated by lead acetate and the lead salts decomposed by hydrogen sulphide, great care being taken to ensure complete decomposition. After filtration, the lead sulphide was again boiled out with a little ammonium sulphide and this filtrate added to the main bulk. After concentrating somewhat, an excess of hot lime water was added to the boiling solution and the precipitated calcium salts filtered off. Of the usually occurring acids all but malic acid are thus precipitated. The insoluble salts were boiled with dilute acetic acid, when all but calcium oxalate dissolved, and determinations of oxalic acid and of tartaric acid made upon these two fractions are recorded below. Succinic acid was also looked for, but proved to be absent. The cooled solution of calcium malate in lime water was acidified with acetic acid and sufficient lead acetate added to precipitate the insoluble lead malate. This was removed, washed and decomposed with hydrogen sulphide by passing a stream of the gas through a suspension of the precipitate in hot water. After filtering off the lead sulphide and boiling vigorously to expel all traces of the gas, the solution was adjusted to a volume of 100 c.c. and an aliquot of 25 c.c. taken for determination by the cerium method.

25 c.c. solution, 40 c.c. decinormal ceric sulphate, 50 c.c. sulphuric acid S.G. 1·5 and 85 c.c. water were refluxed for 30 minutes.

Volume of N/10 ferrous sulphate used in back titration = 5·4 c.c.

∴ Volume of ceric sulphate reduced = 34·6 c.c.

Since 1 c.c. N/10 $Ce(SO_4)_2$ = ·001449 gm. malic acid, this represents 50·14 mgm. malic acid/25 c.c.

or 10·78 per cent. in plant on dry wt. basis.

In a second experiment, an extract was prepared from 1 gm. of the plant powder, treated as above, and made up to a final volume of 100 c.c. A 50 c.c. aliquot of this reduced 35·5 c.c. ceric sulphate solution.

∴ 51·44 mgm. malic acid/50 c.c.

or 11·06 per cent. in plant on dry wt. basis.

Although the agreement was satisfactory in these two determinations carried out upon different quantities of materials, confirmation of the above figures was afforded by the determinations made according to the second method when the ammonium salts of all of the organic acids except malic acid are precipitated by addition of 9 volumes of alcohol to their aqueous solution.

An extract from 2 gm. of plant powder was precipitated by lead acetate and the free acids regenerated by means of hydrogen sulphide. A slight excess of ammonia was then added and the liquid boiled down to a volume of 30 c.c. After cooling, 280 c.c. of 96 per cent. alcohol was added and the precipitate removed. Lead acetate solution in excess of the quantity required to transform the ammonium malate into insoluble lead malate was then added and the precipitate removed, washed and decomposed by hydrogen sulphide, taking the precautions noted above. After vigorous boiling, the resulting solution of malic acid was made up to a volume of 100 c.c. Upon 25 c.c. aliquots of this, determinations of malic acid were made by the cerium method. The amount corresponded to 11·21 per cent. in the plant on the dry weight basis.

It can safely be concluded from the results obtained by these two entirely different methods that, in spite of the difficulties of the problem, the procedures adopted were selective for malic acid.

The mean of the three determinations 10·78 per cent., 11·06 per cent. and 11·21 per cent. is 11·02 per cent.

DETERMINATION OF OXALIC AND TARTARIC ACIDS IN THE RESIDUES FROM THE MALIC ACID DETERMINATIONS.

The calcium salts insoluble in hot lime water were boiled with dilute acetic acid when calcium oxalate remained undissolved. It was filtered off, dissolved in 200 c.c. of hot, dilute sulphuric acid and an aliquot titrated with hundredth normal potassium permanganate.

5 c.c. required 9·4 c.c. N/100 $KMnO_4$.

5 c.c. after reprecipitation required 9·2 c.c. N/100 $KMnO_4$.

This corresponds to 8·59 per cent. in agreement with the figures previously found (mean 8·66 per cent.).

Tartaric acid was determined in the acetic acid solution by precipitation as potassium hydrogen tartrate as previously described. The precipitate required 0·9 c.c. of N/10 NaOH. This corresponds to 0·072 per cent. of tartaric acid agreeing with the figures of 0·064 per cent. and 0·072 per cent. previously found.

Succinic acid was tested for by making a solution of the free acids, regenerated from their lead salts, neutral to litmus and adding neutral ferric chloride solution. No precipitation of ferric succinate was formed indicating the absence of succinic acid.

RESUMÉ OF ANALYTICAL RESULTS.

For convenience, the figures found are here recapitulated. All percentages are upon the dry weight basis. The green plant contained 67·75 per cent. moisture.

Free titratable acidity of the aqueous extract ... ... = 5·15 c.c. N/10 acid per gm.

Ash left on ignition 24·58% } 24·62% } Mean = 24·60%

Acid-insoluble residue (silica, etc.)

1·02% } 1·01% } Mean ... ... = 1·02%

Calcium in the ash as CaO ... ... = 8·19%

Total Ca in the plant ... ... = 1·34%

Potassium in the ash as K_2O ... ... = 5·20%

Alkalinity of the ash: water-soluble = 30·51 c.c. N/10 alkali per gm.

water-insoluble = 10·31 c.c. ,,

Total 40·82 c.c. } 40·12 c.c. } Mean ... ... = 40·47 c.c. ,,

Oxalates as oxalic acid by—

direct method 8·61% } 8·70% } Mean = 8·66%

Indirectly *via* Pb salts during determination of malic acid 8·59%

Malates as malic acid by—
Ca salt method 10·78%
11·06%
NH_4 salt method 11·21%
Mean = 11·02%

Tartrates as tartaric acid by—
direct method 0·064%
0·072%
Indirectly *via* Pb salts during determination of malic acid ... ... 0·072%
Mean = 0·069%

Citrates: absent.
Succinates: absent.

From the protocols of the feeding experiments, recorded in the appendix, it will be seen that the quantity of the fresh plant found to be lethal for rabbits is such as to contain approximately 3 gm. of oxalic acid. The minimum lethal dose of oxalic acid for the rabbit *per os* is given in the pharmacological literature as 2·4 gm. Malic acid is not toxic, in fact it fulfils an important rôle in certain physiological processes in animal metabolism. We were interested to see if any synergistic action was produced by the presence of such large quantities of malic acid along with the oxalic acid in *Psilocaulon absimile*, but such does not appear to be the case.

INTERRELATIONS OF THE ORGANIC ACIDS AND THEIR PHYSIOLOGICAL FUNCTION IN PLANTS.

There has been much speculation as to the rôle played by the organic acids, oxalic, malic, tartaric, citric, etc., in the economy of the plant, and several theories have been advanced.

In the first place it is noticeable that, although the acids concerned enjoy a fairly wide distribution in the vegetable kingdom, it is only in certain genera that they are met with in appreciably high concentration, and such plants are very frequently succulent or xerophytic types. Again it has always seemed perplexing that the quantities of oxalic and malic acids present seem often to be complementary, one rising whilst the other falls during the course of the season, or one being the main representative in a certain species, whilst in another closely allied member of the genus the relative proportions may be quite reversed.

Only recently has an acceptable theory of acid production, harmonizing with experimental data, been put forward. However, it is considered not out of place to give here a very brief summary of earlier views which have led up to the present position.

Mayer (1875, 1878) considered that the organic acids were products of plant respiration and that, under the influence of light, they suffered reduction to carbohydrates. A purely photochemical explanation of their disappearance was also advanced by Spoehr (1913), but, although the acids are sensitive to light of certain wavelengths, there can be no doubt that other factors more intimately connected with metabolic processes come into play in the living plant.

An enzyme with the power of decomposing oxalic acid has been demonstrated in *B. extorquens* by Bassalik (1913), and in the tissues of a wide variety of plants by Staehelin (1919). Light would seem to be capable of exerting a stimulating effect upon oxalate production in leaves.

Kraus (1886a, 1886b) regards the daily fluctuations in acid content of succulents and non-succulents to be a general phenomenon, the accumulation which takes place during the night being due to the incomplete oxidation of these products of respiration. With the return of daylight their oxidative removal is assisted by the higher tension of oxygen set free in the assimilation process and possibly also by the direct action of light itself. With this hypothesis Warburg (1886) is in general agreement. De Vries (1885) emphasized the importance of temperature in regulating the intensity of oxidation.

Oxalates have frequently been regarded as excretory products either incidental or subservient to a useful function by rendering the plant unpalatable and poisonous. They may also exert a regulatory function and some experimental evidence seems to bear out the accuracy of this view. Thus De Bary (1886) was able to show that oxalate production in the fungus *Peziza sclerotiorum* can be stimulated or depressed by including much or little calcium in the culture medium. Wehmer (1897, 1906, 1913) found that *Aspergillus* sp. produces little or no oxalate when grown upon a medium of sugar to which ammonium chloride has been added as a source of nitrogen, but considerable quantities of this acid when ammonium chloride is replaced by peptone. A similar finding is reported by Benecke (1903) who, working with *Zea mais*, found oxalates to be produced in much greater quantity when nitrates took the place of ammonium salts as the source of nitrogen. Amar (1903) showed many carophyllaceous plants could be obtained oxalate-free by allowing the seeds to germinate and grow in a Ca-free medium. An association between incomplete carbohydrate oxidation and the production of oxalic acid has been postulated by many workers, among whom Duclaux (1883) and Wehmer (1891) may be particularly mentioned, and a great deal of experimentation has been directed towards a comparison of the quantities of oxalic acid produced by one or other of the lower organisms when growing upon different individual sugars as the sole source of carbon. It cannot be said that any very clear conclusions of a general nature have emerged from these experiments.

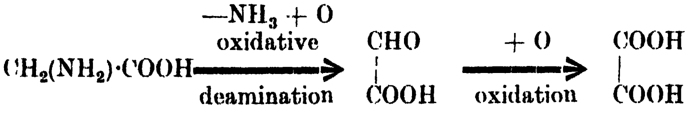
Ruhland and Wetzel (1926, 1927, 1929) have recently brought an entirely new light to bear upon the problem. These authors recognize two types of plants, the " amide " plants and the " ammonia " or " acid " plants. In the former, oxidative deamination of amino-acids leads to the formation of acid amides such as asparagine and glutamine and such plants are characterized by a relatively high content of amide nitrogen and correspondingly low ammonia nitrogen. Acid plants on the other hand differ in containing relatively much ammonia and little amide nitrogen, whilst they are also rich in organic acids. In such plants deamination proceeds so as to form simultaneously organic acids of the malic type and ammonia. The non-nitrogenous residues of deaminated amino-acids are considered to be the source of the oxalic, malic, tartaric and succinic acids found in the vegetable kingdom (c.f. Wetzel, 1927). Ruhland and Wetzel find no correlation between the production of such acids and the degree of respiratory activity and therefore discard the theory that they are to be considered as products of respiratory activity. On the contrary, they were able to demonstrate a close relationship between the degree of deamination and the accumulation of organic acids and of ammonia in such typical acid plants as *Begonia semperflorens* and *Rheum hybridum* Hort. It is of interest that *Psilocaulon absimile* is also rich in ammonium compounds.

Following upon the acceptance of Ruhland and Wetzel's work, many of the earlier experimental findings can be viewed in a new light and many obscure points readily interpreted. The greater production of acids in young leaves during the night time is thus to be linked with the active deamination which is then known to proceed. Similarly the much greater production of oxalic acid by *Aspergillus niger* when peptone replaces sugar in the culture medium is attributable to the more active deamination proceeding in order to supply energy and nitrogen for the metabolic needs of the growing organism. The acid first formed is probably the α-ketonic acid pyruvic acid which Quastel (1925) has shown to occupy a central position with regard to the various lines of synthesis and degradation inherent in the life cycle of the normal bacterial cell. Pyruvic acid may be looked upon as the common exchange medium between the lines of carbohydrate, protein and fat metabolism. Its production from alanine and transformation into malic acid may be represented by the following scheme:—

$$CH_3\cdot CH(NH_2)\cdot COOH \xrightarrow[\text{deamination}]{\substack{-NH_3+O\\ \text{oxidative}}} CH_3\cdot CO\cdot COOH \xrightarrow[\text{reduction}]{+2H} CH_3\cdot CH(OH)\cdot COOH$$

alanine pyruvic acid malic acid

Oxalic acid probably arises from the oxidative deamination of glycine followed by further oxidation to the di-carboxylic acid or by degradation of the acids with longer carbon chains.



$$CH_2(NH_2)\cdot COOH \xrightarrow[\text{deamination}]{\substack{-NH_3+O\\ \text{oxidative}}} \begin{array}{c}CHO\\ |\\ COOH\end{array} \xrightarrow[\text{oxidation}]{+O} \begin{array}{c}COOH\\ |\\ COOH\end{array}$$

glycine glyoxylic acid oxalic acid

Oxalic acid is frequently deposited in various plant organs or specialized cells in the form of the sparingly soluble calcium oxalate, the quantity of which by slow accumulation may often come to represent an astonishingly large proportion of the whole plant. Thus in *Pilocereus senilis*, a member of the cactaceae, potassium oxalate may form between 80 per cent. and 90 per cent. of the total dry substance. *Mesembryanthemum cristallinum*, the lichens *Lecanora esculenta* (66 per cent. calcium oxalate) and *Chlorangium Jusuffii* (65 per cent. calcium oxalate), the bark of the Eucalyptus tree (16 per cent. calcium oxalate) and of Ceylon Cinnamon (6·6 per cent. oxalate) bear further evidence to this accumulation of oxalic acid. The soluble sodium salt occurs in *Salicornia* and *Salsola* species.

Citric and malic acids are frequently deposited as the sparingly soluble calcium salts in a manner analogous to oxalic acid. For example, 3·5 per cent. potassium malate in various *Rheum* species (Castoro, 1902); 8 per cent. expressed as malic acid in *Agave* leaves (Zellner, 1918). In this connection it is of interest to compare the various species of the genus *Mesembryanthemum* (Table I) which have been investigated, but it is also necessary to bear in mind the fact that marked fluctuations in the acid content may occur during the course of the seasons. This latter point is well illustrated by the table reproduced below (Table II) from the work of André (1905). From the toxicological standpoint this seasonal variation is also of direct importance, since such a plant may be highly poisonous at one season and practically innocuous at another. According to Berthelot and André (1886, 1887) the oxalate content of a large number of plants, including *Rumex*, *Amaranthus* and *Mesembryanthemum* species, tends to rise to a maximum in the summer months, declining again towards autumn.

TABLE I.

Distribution of the Organic Acids in *Mesembryanthemum* sp.

	Oxalic Acid.	Malic Acid.	Citric Acid.	Other Constituents.
M. crystallinum L...	Much	Much	Possibly traces	Ash 30–50 % of which 50 % is K (André 1905–06).
M. tortuosum L....	—	—	Present in Raphides with Mg and H_3PO_4	Alkaloid "Mesembrin" also epidermal wax (Hartwich and Zwicky 1914).
M. expansum L.....				
"Channa" of the Hottentots				
M. edule L.........	None	Present	Present	(Berg and Gerber 1896.)
M. linguiforme L....	Little	Much	Little	
M. perfoliatum Mill..	Little	Little	Much	
Psilocaulon absimile..	8·6 %	11·02 %	None	Tartaric acid 0·07 %. (Present work).

TABLE II.

Variation of Content in Organic Acids during the Year; as per cent of Dry Substance (André, 1905).

	Soluble Oxalate.	Insoluble Oxalate.	Malic Acid.
Mesembryanthemum—			
May 26....................	10·53	11·92	3·67
June 13....................	6·16	9·68	4·40
July 1......................	5·29	5·50	10·71
July 22....................	4·86	4·79	—
August 17.................	1·90	2·56	13·83
Sedum azureum—			
May 25....................	0·15	1·67	7·62
June 17....................	0·23	0·25	8·73
June 21....................	0·45	1·62	8·42
July 8......................	Trace	0·74	10·13
July 29....................	Trace	0·35	7·72

TOXICOLOGY OF OXALIC ACID AND OXALATES.

Oxalic acid is a relatively strong acid and its administration is therefore accompanied by some degree of gastric inflammation. The oxalate ion also possesses toxic properties, however, the effects of which are shown equally well by the soluble potassium and sodium salts as by the free acid; calcium oxalate, on account of its low solubility is poorly absorbed and is therefore quantitatively much less toxic. Chickens are said to be practically immune to oxalic acid poisoning, if administered orally, on account of the high proportion of calcium present in the contents of their intestines.

The toxic effects of oxalate ingestion may be summarized briefly as follows:—

(1) A local inflammation, present even when the acid is administered in dilute solution.

(2) Muscular twitching or tetany, accompanied by other nervous symptoms, due to the removal of calcium ions from the system and an upset of the base balance CaMg/NaK.

(3) Lowered coagulability of the blood owing to the decrease in calcium ions.

(4) Lesions in the excretory organs, kidney, etc., owing to the deposition in the cellular substance of hard, crystalline concretions of calcium oxalate.

In the experiments carried out upon rabbits during the course of the present work, it was found that a dose of 4·4 gm. of sodium oxalate (equivalent to 2·9 gm. oxalic acid) given per os to a 3 kilogram rabbit was sufficient to cause death in about 1 hour, the chief symptom being muscular weakness and tremor, followed by violent convulsions as death took place. Administration of a solution of the regenerated lead salts of the organic acids from 33 gm. dried *Psilocaulon absimile*, containing approximately 2·9 gm. oxalic acid, produced similar results, death taking place rather more quickly when the unneutralized fluid was given than when previously neutralized by sodium hydroxide.

Chronic oxalic acid poisoning through the ingestion of *Oxalis cernua* the "South African wood-sorrel" or "Soursobs" has been described by Bull (1929), who found farm animals in Australia to be affected by feeding upon this plant. A period of 6 to 8 weeks was required before the symptoms became serious or fatalities occurred.

Affected animals lost control of the fore-quarters or hind-quarters becoming unable to rise. Tonic spasms of the muscles of the forelegs and neck were noticed. In some cases death was sudden, whilst other animals lingered on for a greater or lesser period. On post-mortem examination the kidneys were found to be pale in colour, containing some fibrous tissue in the cortex; extensive degenerative changes were seen in the tubules, whilst scattered throughout the cortex and boundary zone numerous refractile deposits of crystalline calcium oxalate were found.

Continued ingestion of *Psilocaulon absimile* in sub-lethal doses would presumably lead eventually to the same result.

In the Willowmore cases of suspected subacute *Psilocaulon* poisoning in Angora goats the affected animals exhibited the following symptoms:—Cachexia, extreme weakness, anaemia, chronic diarrhoea and inappetence. The post-mortem revealed anaemia, hydroperitoneum, hydrothorax, hyperaemia of the lungs, liver and kidneys and a severe acute catarrhal gastro-enteritis.

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Section IV.

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Onderstepoort Journal of Veterinary Science and Animal Industry, Volume I, Number 2, 1933.

Studies on the Photosensitisation of Animals in South Africa.

I. The Action of various Fluorescent Dye-stuffs.

By J. I. QUIN, D.V.Sc., Veterinary Research Officer, Onderstepoort.

INTRODUCTION.

UNDER the title "Studies on the Photosensitisation of Animals in South Africa" it is intended to report on a series of different investigations which have been undertaken in the first place in an endeavour to elucidate the problem of "Geeldikkop" amongst small stock in South Africa. This disease has been in existence here for many years and has frequently been mentioned in the Reports of the Colonial Veterinary Surgeon of the Cape of Good Hope from 1894-1906.

In the Seventh and Eighth Reports of the Director of Veterinary Research (1918), Theiler, who carried out various experiments in different localities, describes the disease in detail. Furthermore, he definitely showed that the "dubbeltjie" (*Tribulus* species) could under certain conditions provoke geeldikkop, a belief which many stock-owners had held for years. The disease, however, occurs spasmodically and may be very difficult to reproduce as shown by Theiler's work, and also from investigations conducted subsequently by the author. According to Theiler, geeldikkop is caused exclusively by *Tribulus terrestris* in the flowering stage. It should, however, be pointed out that the term "geeldikkop" is rather misleading when used in this restricted sense, seeing that the symptom complex, viz., a generalised icterus accompanied by swelling of the head may be encounterd on may different pastures in South Africa where *Tribulus* is practically unheard of. In such cases it is frequently referred to as geeldikkop, dikkop, dikoor, geelsiekte, terms which must be regarded as purely symptomatic in their description. In fact, from an examination of affected animals, the sudden onset of the condition and the post-mortem appearances, one is forced to the conclusion that the underlying factors at work are of a similar nature in each instance. The object of these investigations was to gain a better understanding of these fundamental principles. The importance of one factor at least, is, however, realized, viz., the influence of sunlight, since the disease is definitely associated with photosensitisation. Thus affected animals always seek shade, while the subcutaneous swelling and necrosis of the skin is always localised to the exposed and unpigmented parts, viz., over the face and ears. The icterus, on the other hand, is much less clearly understood. There is, however, some damage of the liver as shown by the necrosis of hepatic cells and proliferation of the bile capillaries. Whether this is due to some specific toxic agent in the plants, or to some derangement in the metabolism of the plant or the animal body or to changes in both, it is exceedingly difficult to determine as yet. The view that some metabolic disturbance is at the root of the trouble is favoured for the following reasons:—

(*a*) Animals may sicken very suddenly on a pasture which normally is considered excellent for small stock, e.g. on the Karroo where hundreds of thousands of animals are kept and where the *Tribulus* plant forms a substantial part of the diet.

(*b*) A condition similar in all its aspects may at times be encountered on lucerne paddocks and where *Tribulus* can be ruled out.

(*c*) On grass veld, e.g. the Transvaal highveld and in the northern Orange Free State, the condition may suddenly appear and cause heavy losses in sheep. Here again *Tribulus* can be excluded.

(*d*) Farmers in certain parts of the Karroo have reported outbreaks of so-called "geeldikkop" in mid-winter, when no *Tribulus* plants are to be found (also mentioned by Theiler in his report).

It thus seems evident that *Tribulus* cannot be the only cause of geeldikkop, and that a number of widely different plants will have to be considered.

With regard to *Trifolium*, of which several species are either cultivated or found growing wild in South Africa, no complaints have been made in this respect, although Fröhner states that in Europe several species of *Trifolium* have from time to time caused photosensitisation and icterus in animals.

Buckwheat poisoning commonly noticed in unpigmented animals in Europe, is not a serious complaint in South Africa, seeing that very little buckwheat is grown. Experimentally, however, it has been shown that the typical symptoms noted in other countries can be producd in animals under South African conditions when *Polygonum fagopyrum* plants are fed. Similarly it has been demonstrated experimentally that different species of *Hypericum*, viz., *H. ethiopicum* and *H. leucoptychodes* found in South Africa, may cause photosensitisation of sheep. In these cases the symptoms noted correspond exactly with those described in European countries are due to *Hypericum perforatum* and *Hypericum crispum*, viz., marked photosensitisation of unpigmented animals accompanied by large subcutaneous swellings of the head and ears, followed by necrosis and sloughing of the affected skin. Both in buckwheat and *Hypericum* poisoning the complete absence of icterus is an interesting point, seeing that in South Africa, outbreaks of geeldikkop occurring on the pasture are practically always characterised by an icterus of an intense nature.

On account of the great difficulty experienced in attempting to produce cases of geeldikkop experimentally, especially under laboratory conditions away from naturally occurring outbreaks, it was decided to induce photosensitisation by various other means. It was hoped that in this way a clinical picture simulating that of the true disease, could perhaps be produced. It may be mentioned that the disease appears and disappears in a most insidious manner, and at its worst only lasts for a comparatively short while during the summer months. Furthermore, poisonous *Tribulus* plants, once they are removed from their habitat seem to lose their toxicity without delay. It was for these reasons that one was forced to resort to other means of provoking the disease. Seeing that sheep and goats are practically the only animals naturally affected, most of the experiments were conducted on these animals. In a few cases white rabbits were also tried, but, as a rule, they were unsatisfactory on account of their marked sensitivity to the heat rays in strong sun-light. Many of them died after a few hours exposure from symptoms of shock probably due to "heat stroke".

In a previous paper by Quin (1931), the photosensitising influence of haematoporphyrin on sheep and goats was reported upon. In that work it was shown that injections of small amounts of haematoporphyrin (0·5 gm.) provoked an almost immediate and intense photosensitivity in unpigmented sheep and goats exposed to sunlight. The subcutaneous oedema at first, and the necrosis and sloughing of the skin afterwards, were both very marked. In all these cases, however, icterus was completely absent, as the liver appeared to maintain its normal function. Otherwise the symptoms were wholly in accordance with those of geeldikkop. Presumably haematoporphyrin only causes a simple direct photosensitisation without damaging protected organs and tissues, as no evidence of this was shown at post-mortem on animals that were killed. In geeldikkop, on the other hand, liver function seems to be definitely deranged as well, i.e. there must be some icterogenic factor operating at the same time that the animal becomes photosensitive. Seeing that haematoporphyrin did not provoke any icterus, it was decided to ascertain the effects of various fluorescent chemical substances on sheep and goats. In the earliest work in photosensitisation Raab, Jodlbauer and Busck showed that such substances as erythrosin and rose bengale when injected into rabbits produced irritability, well marked oedema, skin necrosis and loss of hair when exposed to sunlight.

The following experiments were, therefore, carried out on young Merino sheep 12-15 months old. The animals were first closely shorn, especially the head and along the back, and then kept exposed in sunlight for several hours every day.

I. EXPERIMENTS WITH DYE-STUFFS IN THE FLUORESCEIN GROUP.

(*a*) TETRABROMFLUORESCEIN OR EOSIN.

One gram eosin was dissolved in 20 c.c. saline and injected intrajugularly into a sheep. Within a few seconds all visible mucous membranes and also the exposed skin assumed an intense pink-red colour. Ten minutes after the injection the sheep became extremely restless in the sunlight. There was marked flinching of the body, shaking and scratching of the head and ears. Due to the severity of the attack of photosensitisation the animal was placed in the stable half an hour afterwards. The symptoms now soon passed off, and within 3 hours after injection the sheep was feeding quietly. The urine was of an intense red colour throughout the day due to elimination of the eosin. The next day the animal was again placed out in the sun. Photosensitisation was very slight, and the ears were slightly swollen. The blood serum now was quite clear and colourless. Subsequently two injections of 0·5 gm. eosin daily, produced only

Fig. 1. Acute photosensitisation following injection of Eosin.

slight irritation over the head when the sheep was placed in the sun. Another injection of 1 gm. eosin given on the 6th day caused extreme photosensitisation and rapid swelling of the ears (see fig. 1). The animal, after being kept in the stable for 2 days, seemed completely recovered. The head, ears and back of the same sheep was then stained thoroughly with a concentrated watery solution of eosin, and shortly afterwards 1 gm. eosin again injected intravenously. On now being placed in strong sunlight the animal showed no signs of photosensitisation, clearly indicating that the eosin applied to the skin and wool had prevented the harmful rays of the sun from affecting the body. It was then decided to ascertain the effect of oral administration of oesin. One Angora goat was dosed 10 gm. eosin in 1 litre water. The faeces rapidly became stained with eosin and a mild diarrhoea was noted for a few days. There were, however, no signs of photosensitisation.

From these experiments it was thus seen that eosin when injected intravenously produces a very marked sensitisation to sunlight, followed by oedema of the ears and face in sheep. No signs of icterus were, however, noticed. Eosin when applied to the skin and wool efficiently acts as a protection from the harmful rays when at the same time eosin is injected intravenously. Furthermore, it has been shown that the oral administration of eosin does not lead to any photosensitisation, probably due to the rapid passage through the alimentary tract and the accompanying poor absorption.

(b) TETRAIODOFLUORESCEIN OR ERYTHROSIN.

One gram of erythrosin in saline injected intravenously into a sheep, caused marked sensitisation and scratching of the ears within 3 minutes after being placed out in the sun (see fig. 2). The animal was then returned to the stable. This caused a rapid disappearance of the symptoms. A subsequent injection of a further 0·5 gm. erythrosin again provoked intense sensitisation accompanied by oedematous swellings of the head, ears and also of the skin round the anus. When the swellings had subsided, with the animal kept stabled, the head and back was thoroughly coloured with a strong solution of erythrosine. After that an intravenous injection of 1 gm. erythrosine was given and the sheep placed out in the sun. No symptoms were shown indicating that the colouring on the skin had caused protection against the sunlight. As in experiment 1A it should be mentioned that no sign of icterus was ever shown, the serum remaining water clear after elimination of the dye-stuff.

Fig. 2. Acute photosensitisation following injection of Erythrosin.

(c) TETRACHLORTETRAIODO-FLUORENSCEIN OR ROSE BENGALE.

As in the previous experiments 1 gm. rose bengale was injected intravenously into a sheep. After being exposed in the sun for 8 minutes the animal showed marked irritability, scratching and flinching and frequently sitting down on the haunches, or dragging the hindquarters along the ground (see fig. 3). When placed in the stable the animal still showed symptoms 4 hours afterwards. The next morning the ears were swollen, although the sensitiveness had passed off. Two subsequent injections of 0·5 gm. each, again provoked marked symptoms when the animal was placed in the sun. After the symptoms had subsided, the head and back was coloured with a strong solution of rose bengale and 1 gm. injected intravenously. No symptoms were shown when the animal was exposed in strong sunlight. Three weeks after the initial injection the skin over the back was felt to be extremely hard and causing a peculiar stiff gait. A few days later, extensive sloughing of the affected skin set in, leaving a raw bleeding surface in some parts (see fig. 4). Complete recovery took place in time, accompanied by a fresh growth of wool. No signs of icterus were ever noticed.

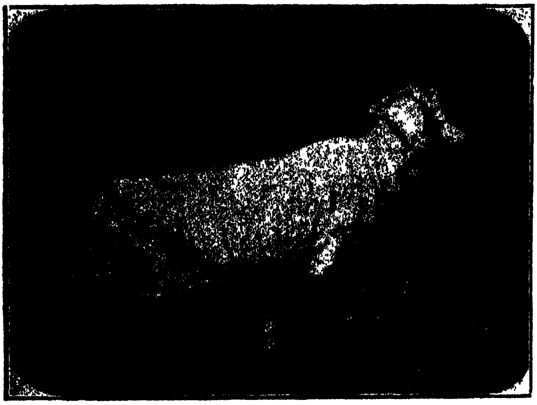

Fig. 3. Acute photosensitisation following injection of Rose Bengale.

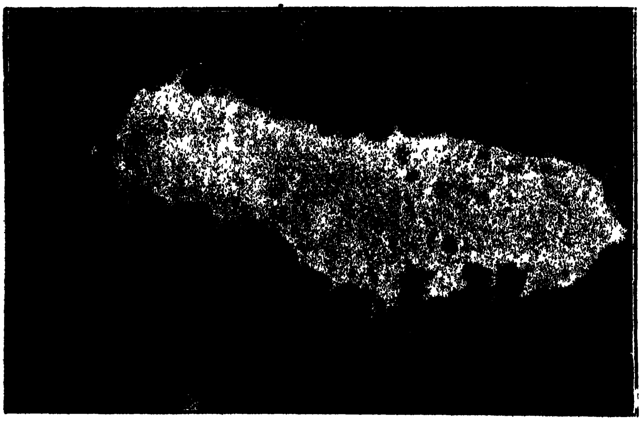

Fig. 4. Chronic skin lesions following injection of Rose Bengale.

Although the action of the three fluorescein dyes tested, was essentially the same, rose bengale produced stronger photosensitisation than the other two. From these experiments it thus became clear that although the fluorescein dyes produced striking photosensitisation, no icterus was ever shown. In this respect, the symptoms provoked seemed to be identical with those produced by haematoporphyrin.

II. EXPERIMENTS WITH DYE-STUFFS IN THE ANTHRACENE GROUP.

For this experiment dichloranthracene-disodium-sulphonate was selected. One sheep was injected intravenously 0·3 gm. the first day without showing any symptoms. On the second day 1·5 gm. was injected and on the third day 0·95 gm. The animal was kept under observation for several days but as no symptoms developed, it was discharged.

III. EXPERIMENTS WITH SUBSTANCES IN THE ACRIDIN GROUP.

In this experiment acriflavin was selected. One sheep injected 0·01 gm. acriflavin intravenously showed no symptoms when placed in sunlight. The following day 0·05 gm. was injected. Within 15 minutes the animal was markedly sensitive, running about the paddock, or repeatedly lying down and then rising. It was then placed in the stable. Two subsequent injections again caused marked symptoms. The irritation of the head was intense, as shown by the continuous rubbing against the fence. The symptoms, however, soon cleared up after the last injection. At no time were there any signs of icterus to be seen, the serum also remaining water clear.

IV. EXPERIMENTS WITH SUBSTANCES FROM THE THIAZIN GROUP.

(A) Methylene Blue.

One sheep injected intravenously with 1 gm. methylene blue showed marked sensitisation in the sunlight 6 minutes after the injection. At times there was marked flinching of the body, which suddenly gave way and causing the animal to assume a crouching position or even to crawl along on the abdomen. These symptoms rapidly subsided when the animal was placed in the stable. When the sheep was again exposed in sunlight 4 hours after the injection, no symptoms were shown. This was probably due to the effective elimination of the dye from the body within a short period. Subsequent injections of methylene blue produced other toxic symptoms, e.g. fairly marked haemolysis, dullness and loss of appetite. The animal died on the 8th day after the first injection, after marked loss of condition and signs of toxaemia. Post-mortem examination revealed pulmonary oedema and cardiac dilatation. There was no icterus.

An Angora goat injected with 1 gm. methylene blue intravenously showed extreme photosensitisation which lasted only for a period of 15 minutes and then suddenly disappeared in spite of the animal being kept out in the sun. A peculiar symptom was the marked vomiting shown immediately after the injection. This, however, also soon passed off. Subsequent injections of methylene blue only produced slight photosensitisation lasting a few minutes. Haemolysis was again noticed, although this did not cause death, neither was icterus noticed at any time. The animal was discharged from experiment after two weeks.

From the above experiments it is thus clear, that methylene blue can produce very strong photosensitisation, which, however, is of short duration, probably due to rapid elimination of the dye. In addition, methylene blue also acts haemolytically on the red blood cells, and may also cause a toxaemia in other respects.

(B) THIONINE.

One Angora goat was injected 1 gm. thionine intravenously. The animal became acutely photosensitive within a few minutes (see fig. 5). There was marked flinching and scratching until the goat was placed in the stable, when the symptoms soon passed off. Subsequent injections again produced sensitisation. The elimination of the dye, however, was very rapid as noticed from the clearing of the serum, and the transitory nature of the symptoms. Slight haemolysis was noticed on one occasion, although blood was drawn and centrifuged regularly every day. Another goat was dosed 10 gm. thionine in 1 litre 5 per cent. Na-bicarbonate solution and placed in the sun. Symptoms of photosensitisation were marked three hours after dosing. The animal, however, died during the night and was decomposed the following morning.

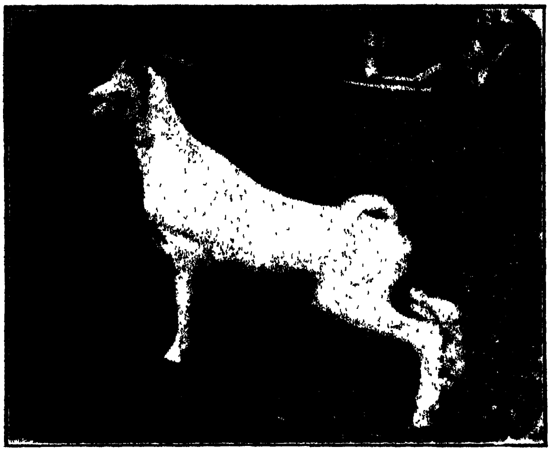

Fig. 5. Acute photosensitisation following injection of Thionine.

Thionine may thus be regarded as capable of producing a well-marked but transitory photosensitisation similar to that caused by methylene blue, although it seems to be less toxic than the latter dye-stuff.

(C) METHYLENE VIOLET.

One Angora goat injected intravenously with 1 gm. methylene violet showed marked photosensitisation almost immediately it was placed out in the sun. There was marked flinching and scratching until the animal was returned to the stable, when the symptoms subsided. A subsequent injection on the following day caused death from shock-like symptoms. It thus seems that, although methylene violet produces well-marked photosensitisation, it is even more toxic than methylene blue

(D) METHYL VIOLET.

To ascertain whether a non-fluorescent substance such as methyl violet could produce photosensitisation, one Angora goat was injected intravenously on three successive days with 1 gm. The animal, although it was kept out in the sunlight, never showed any signs of photosensitisation. There were, however, signs of abdominal pain and loss of appetite on the second day. As the condition did not improve the animal was killed on the 7th day. No specific changes were noted at post-mortem examination, except that there was very little food in the digestive tract.

V. EXPERIMENTS WITH QUININE SALTS.

Seeing that solutions of quinine salts fluoresce in violet and ultra-violet light, it was decided to ascertain whether photosensitisation could be induced by injections of quinine compounds. One Angora goat was injected intravenously with 0·3 gm. quinine hydrochlorate in water, without becoming sensitive when placed in the sun. A further injection of 0·5 gm. given the following day caused a well-marked haemoglobinuria without any symptoms of photosensitisation. The serum soon cleared up and the animal became apparently completely normal again 48 hours after the last injection.

Another goat was dosed with 10 gm. quinine sulphate in 1 litre water. The animal showed a transitory dullness but no signs of photosensitisation were noticeable. The serum remained water clear.

It thus appears that the quinine salts used in these experiments do not readily provoke photosensitisation, although haemolysis and haemoglobinuria may be well marked.

SUMMARY.

In an attempt to produce a clinical picture simulating that of geeldikkop in sheep, viz., generalised icterus accompanied by acute photosensitisation, various fluorescent dye-stuffs were administered to Merino sheep and Angora goats which, after being closely shorn, were kept out in strong sunlight, and carefully observed for any signs of light sensitivity.

In the fluorescein group, eosin, erythrosine and rose bengale were injected into sheep and goats. Photosensitisation in each case was observed within a few minutes, flinching and scratching becoming so marked that the most abnormal body attitudes were assumed by the animal. When the animal was protected from sunlight either by stabling or by pigmenting of the exposed skin, no untoward effects were shown by the injected dyes. In chronic cases marked sloughing of the affected skin took place, accompanied by a new growth of skin and wool underneath. In no case was there any suspicion of any derangement of the internal organs and icterus was constantly absent.

Dye-stuffs taken from the Anthracene group gave no positive results of photosensitisation.

From the Acridin group, acriflavin was found to produce marked light sensitivity which, however, soon passed off.

In the Tiazin group methylene blue was found to produce very marked sensitisation of short duration and accompanied by other toxic effects, e.g. haemolysis. Similarly thionine and methylene violet produced marked symptoms of photosensitisation, although again accompanied by other toxic effects. A non-fluorescent substance such as methyl violet, on the other hand, showed no effect of photosensitising an animal.

Experiments carried out with certain quinine salts showed that, although they were fluorescent in ultra violet light, no photosensitisation resulted when they were injected into sheep. A direct toxic effect was, however, noted in the form of haemolysis and accompanying haemoglobinuria.

From these experiments it is thus clear that, as with haematoporphyrin, marked photosensitisation can be provoked in Merino sheep and Angora goats by the injection of different fluorescent dye-stuffs, and subsequent exposure of the animals to sunlight. The condition, however, differs from true geeldikkop in that the constantly occurring generalised icterus is absent.

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Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 1, Number 2, 1933.

Studies on the Photosensitisation of Animals in South Africa.

II. The presence of a lethal factor in certain members of the plant genus *Tribulus*.

By CLAUDE RIMINGTON, M.A., Ph.D., B.Sc., A.I.C., Research Fellow under the Empire Marketing Board, and J. I. QUIN, D.V.Sc., Veterinary Research Officer, Onderstepoort.

DURING the investigation of outbreaks of " geel-dikkop ", " yellow-thick-head " or " tribulosis " in sheep due to the ingestion of certain species of the plant genus *Tribulus*, the observation was made by one of us (Quin, 1928) that dosing the expressed juice of these plants to healthy sheep resulted in their death. The most outstanding symptom was a discoloration of the conjunctivae, the blood vessels having a chocolate-brown colour. Post-mortem examination revealed a similar discoloration throughout the body; the blood was dark brown in colour and on spectroscopic examination showed a pronounced absorption band in the red, at about 630 mμ. It was strongly suspected that the abnormal pigment present was methaemoglobin.

No signs of icterus or of photosensitvity, the characteristic outward symptoms of geel-dikkop, were observed following administration of the juice of *Tribulus* plants.

Although, symptomatically, there would appear to be little resemblance between geel-dikkop and poisoning by *Tribulus* juice, it is clear that the plant must contain a lethal factor which under these particular conditions is most effective. The problem therefore on its own merits was worthy of chemical investigation, but the hope was also entertained that its elucidation might help to throw some light upon the naturally occurring disease.

The investigations detailed below have been made with material from three sources, namely, a *Tribulus* species growing in the poison garden at the Laboratory (referred to hereunder as " Onderstepoort *Tribulus* "), a quantity of sun-dried *Tribulus* plants brought from the farm estate " Melton Wold " in the Victoria West District, Cape Province, which was the scene of an outbreak in 1932 (Melton Wold *Tribulus*), and a consignment from a farm in the Northern Transvaal (N. Transvaal *Tribulus*). In view of the uncertainty existing among botanists as to the deliniation of species in the genus, it is felt advisable to adhere for the present to the designations given rather than to quote botanical names which may shortly require revision. Specimens of the actual plants are preserved in the Onderstepoort Herbarium and also in the Herbarium of the Division of Plant Industry, Pretoria.

FEEDING TESTS WITH *Tribulus* JUICE AND WITH AQUEOUS EXTRACTS OF THE DRIED PLANTS.

A repetition of feeding tests with expressed *Tribulus* juice entirely confirmed previous observations of Quin (1928) made in the field during an outbreak of " geel-dikkop ". Quantities of from 4½ to 5 kilos of freshly-gathered Onderstepoort *Tribulus* plants were squeezed in a hand-operated press and the dark green juice collected. The yield averaged 1½ litres from 5 kilos. The juice contained much chlorophyll and protein and was slightly acid in reaction; it was noticed that fermentation, or some type of decomposition accompanied by gas formation, set in very rapidly (1 to 1½ hours) when the liquid was allowed to stand at room temperature. When added to a drop of blood, a drop of the juice caused immediate formation of a pigment similar to methaemoglobin. If dosed as soon as collected to a sheep (by stomach tube) death usually followed within some hours, the blood vessels of the conjunctivae having a chocolate-brown colour and the blood of the animal exhibiting, when examined spectroscopically, a marked absorption band with its centre located at about 630 mμ. Post-mortem examination showed the whole of the blood and organs to be dark brown in colour. The myohaemoglobin was, apparently, not affected, neither were there any other macro-or microscopic changes to be noticed. There was no haemolysis.

Juice which had been desiccated by exposure in shallow trays showed the same effects and had apparently lost little of its potency, since the equivalent of 2¼ kilos of fresh plant when stirred up with water and further treated chemically yet killed a sheep with the typical symptoms in 3½ hours.

That the abnormal pigment produced is actually methaemoglobin was shown by examining the behaviour of the blood when reducing agents were added and by comparison with an authentic specimen of methaemoglobin. Sodium hydrosulphite when added to either sample caused an immediate disappearance of the brown colour and a change of the absorption spectrum to that of reduced haemoglobin which could be re-oxygenated to oxyhaemoglobin by shaking with air. Haematin, with which methaemoglobin might possibly be confused, is transformed by reducing agents to haemochromogen but no sign of the absorption spectrum of this pigment was evident.

Aqueous extracts of about 1 kilo of dried plant were found to produce the same effects as did *Tribulus* juice. Our usual procedure was to allow the plant powder to macerate overnight in 2 to 3 litres of distilled water and then to squeeze off the liquid in the press.

All these specimens of dried *Tribulus* when tested for the presence of alkaloids, by extraction with Prolius' solution, gave negative results.

THERMOSTABILITY OF THE TOXIC SUBSTANCE.

Experiments were next made to ascertain whether the toxic substance present in *Tribulus* juice would withstand heating.

By immersion of the flask containing the juice in a water bath heated to 65-70° it was found that complete precipitation of the protein material occurred when the temperature of the liquid reached 61°. When immediately cooled, filtered and dosed to a sheep, such juice was found to be nearly as toxic as the original material causing death with methaemoglobin formation. However, autoclaving the fresh plant for one hour before squeezing destroyed its toxcicity.

Other observations recorded were as follows:—

Treatment of Material Prior to Drenching.	*Result.*
Juice from 2·5 kilos fresh *Tribulus* steamed for 30 minutes..	Negative: No symptoms.
1 kilo dried *Tribulus* in 5 l. water steamed for 30 minutes, then left to macerate overnight. Squeezed off and dosed	Negative: No symptoms.
1 kilo dried *Tribulus* in 5 l. water left macerating overnight, then squeezed off and the extract boiled for 5 minutes before dosing	Positive: Death with typical symptoms.
As above, but boiling continued until extract reduced to 1/3 of its bulk	Transitory methaemoglobinaemia. Animal recovered.
1 kilo dried *Tribulus* in 3 l. of 0·5 aqueous formalin solution left macerating overnight. Squeezed off and dosed	Symptoms appeared 24 hrs. later. Animal listless and staggering about: conjunctivae brown. Died shortly afterwards with typical methaemoglobinaemia.
1 kilo dried *Tribulus* in 3 l. of water left macerating overnight. Squeezed off and mixed with 500 cc. whole sheeps' blood immediately before dosing. Colour of liquid, coffee brown	Negative: No symptoms.

NON-EXTRACTION OF THE TOXIN BY ALCOHOL.

One kilo of Onderstepoort *Tribulus* (dried and ground) was extracted overnight at room temperature with 4 litres of 96 per cent. alcohol. After squeezing in the press, the residue was re-extracted for some hours with another 2 litres of alcohol and this extract added to the first. The combined extracts were concentrated by distillation *in vacuo* at a temperature not exceeding 50° until much reduced in bulk, when concentration was continued at room temperature in front of an electric fan. The syrup was taken up in 500 c.c. of water filtered from chlorophyll . . . . and drenched to a sheep. The result was *negative*. The plant material remaining after the alcohol extraction was therefore spread out to dry and then extracted overnight by 4 l. of water. This extract when dosed to a sheep caused the typical methaemoglobinaemia within a few hours, the animal dying overnight.

The toxic factor does not, therefore, pass into the 96 per cent. alcoholic extracts.

In a similar manner it was shown that an 80 per cent. alcoholic extract possessed a very slight activity but that the residual plant material was inactive. Attempts to extract the toxic factor by 75 per cent. alcohol and then to precipitate it after various purifications of the extract, by addition of excess of alcohol met with no success.

The apparent disappearance of the "toxin" when concentrations of alcohol less than 96 per cent. were used to extract the plant seemed to us very baffling at the time although in view of our later findings, this behaviour is readily understandable. That an enzyme was the toxic agent, was considered to be definitely excluded by the undiminished toxicity of expressed plant juice which had been heated to 60-65° for as long as one hour.

NON-PRECIPITATION BY LEAD ACETATE OR MERCURIC CHLORIDE.

Following our usual system in the examination of toxic plants, we next endeavoured to precipitate the substance from aqueous extracts, prepared by maceration overnight, by lead acetate, mercuric chloride or phosphotungstic acid. In the former case, clear-cut results were obtained showing that neither with neutral nor with basic lead acetate nor with basic lead acetate and ammonia was an insoluble compound formed; the toxicity was preserved in the filtrate from these substances. It would appear, then, that the toxic substance was not acidic in character nor is its glucosidal nature likely although many glucosides do resist precipitation even by lead acetate and ammonia. Experiments with mercuric chloride were equally convincing, thus casting doubt upon the basic character of the toxin. Complete activity was retained in the filtrate after lead and mercury precipitation. Less satisfactory results followed the use of phosphotungstic acid as, probably owing to the high concentration of acid required when using this reagent, the toxin was invariably destroyed.

Of the substances known to be capable of producing methaemoglobin when fed to animals, by far the largest class comprises organic nitro-compounds such as nitrobenzene, nitroglycerine and picric acid, or amino compounds derived therefrom by reduction of the nitro group. Toluylendiamine may be cited as a typical example of such substances. Inorganic nitrites are, of course, known to produce the transformation of haemoglobin into methaemoglobin *in vitro* as does also the bitter pincipal podophyllotoxin.

Reviewing our results, it appeared that a simple organic substance could be definitely excluded on account of the insolubility of the lethal factor in 96 per cent. alcohol, nevertheless attempts to demonstrate its presence in the inorganic fraction failed. The apparent disappearance of the toxin when alcohols of lower concentration than 96 per cent. were used and its reactions towards heating were also confusing phenomena.

An examination was then made of an extract cleared by means of lead acetate and by mercuric chloride in the hope that after the removal of such quantities of extraneous material, further *in vitro* tests would help to throw light upon the problem. As an index of activity the action upon freshly-drawn sheep's blood was adopted since it appeared to us that the *in vivo* and *in vitro* actions were certainly related.

The following observations were recorded:—

1. When added to diluted blood, immediate formation of methaemoglobin occurs if the pH is about 4·6. Acid haematin was not formed at this acidity, neither was conversion to methaemoglobin appreciable at higher pH values.
2. At a pH of 4·6 the ferrous ion, added as ferrous ammonium sulphate, is immediately oxidized to the ferric state, shown by the blood-red colour with potassium thiocyanate.
3. Acid potassium permanganate is reduced.
4. Acid potassium iodide-starch solution is coloured intensely blue (oxidation).
5. The metaphenylenediamine reagent gives a brown colour.
6. The Griess-Ilosvay reagent (naphthylamine-sulphanilic acid in acid solution) gives an immediate, intense, rose-red colour.
7. The brown ring test with ferrous ammonium sulphate and concentrated sulphuric acid was positive.

From these reactions it appeared that the oxidising agent, the toxic factor converting haemoglobin to methaemoglobin was none other than inorganic nitrite. Reactions 5 and 6 which depend upon the diazotization of an amino body are specific for nitrites. A solution of sodium nitrite gave all the above tests in a manner identical with the plant solution. When reviewed in this light our preliminary experiments were seen to be in perfect harmony with such a conclusion. The only point which at first caused us doubt was that nitrites are only encountered in traces in plants and it seemed highly improbable that a kilogram of dried plant should contain sufficient nitrite to kill a fully grown sheep when given by the mouth.

Feeding tests, which will be considered in more detail below, showed that the lethal dose of sodium nitrite is about 2 gm. *per os* for a sheep weighing approximately 20 kilos.

A consideration of the form in which nitrite could be present in these plant extracts enables the possibilities to be limited to three, namely:—

1. That it is present in solution as free, inorganic nitrite.
2. That it is present in combination with glucose as an extremely unstable glucoside such as the nitrite-glucoside reported to be present in the leaves of *Erythrina* sp.
3. That it is formed from some other precursor.

On account of its reactivity, nitrite is not easily determined in a plant extract rich in proteins, amino acids, etc., however, preliminary experiments showed quite clearly that its amount varied considerably in different extracts and in one and the same extract under different conditions, thus ruling out possibility number 1.

To quote only one example, three extracts prepared on different occasions by macerating 200 gm. lots of dried *Tribulus* overnight with 800 c.c. of water were found to contain quantities of nitrite equivalent to 1·6, 0·15 and 0·31 gms. of $NaNO_2$ per kilogram of plant. Extracts made with boiling water contained no more than traces of nitrite.

That nitrite could be present in the plant in the form of an unstable glucoside was a possibility considered since Weehuizen (1907) reported the occurrence of such a glucoside and of an enzyme decomposing it with liberation of nitrous acid in the leaves of an unidentified *Erythrina* species, an observation subsequently substantiated by Betting (1909).

For several reasons, however, the occurrence of a nitrite-yielding glucoside in our *Tribulus* samples appeared to us unlikely in view of our experimental findings. Although nitrite is absent from the dry, powdered plant material, it is rapidly formed, and in considerable quantity, when such preparations are soaked in water in a closed vessel. In one instance 100 gm. plant powder (Onderstepoort *Tribulus*), and 1 litre of water to which had been added 1 c.c. of cloroform was left in a closed jar at room temperature for 60 hours. When opened the smell of nitrous oxide was apparent and a strip of starch-iodide paper held in the vapour was immediately coloured deep blue. An attempt was made to determine the quantity of nitrite (or nitrous acid) still in solution by filtering, decolorising the filtrate by shaking with animal charcoal (proved to contain no nitrite) and carrying out a colorimetric determination upon the filtrate by means of the Griess-Ilosvay reagent. Reckoned as sodium nitrite, 0·8 gm. was found to be present.

The most likely source of this nitrite formed in extracts of *Tribulus* or in the press juice of the plant appeared to be inorganic nitrates, which are known to be frequently, although not invariably, present in plant tissues. The only possible objection to such an hypothesis was the relatively large quantity demanded by the formation of so much nitrite. A reducing enzyme or enzyme system capable of bringing about the reduction of added nitrate has been shown to be present in quite a number of plant tissues (Anderson 1924).

It became imperative to carry out determinations of the nitrate present in our *Tribulus* samples, and for this purpose the method proposed by Strowd (1930) was finally adopted, the values being checked later by the highly specific phenolsulphonic acid colorimetric method.

DETERMINATION OF NITRATE IN DRIED *Tribulus*.

All the preliminary work was done using samples taken from a large stock of ground, dried *Tribulus* (from all three localities in order to check the reproducability of the results).

Preparation of the extract. Since it was important that all enzymic changes should be eliminated, extracts were made by adding a weighed sample of the plant powder to a known volume of boiling water, maintaining ebullition for 2 or 3 minutes and then allowing the mixture to stand, plus a few drops of toluol, in a stoppered flask overnight. A satisfactory proportion was to take 20 gm. of plant and 200 c.c. of water.

The extract was strained off through fine muslin and the residue squeezed in a press, after which it was quantitatively transferred to another portion of 200 c.c. of boiling water and the extraction process repeated. The extracts were combined and the resulting volume measured. Aliquots of 50 c.c. were found to be satisfactory for the determination of nitrate by the Devarda alloy method.

Two 500 c.c. pyrex flasks were fitted with kjeldahl splash-bulb distillation traps and these, in turn, connected to two vertical water-cooled condensers. The delivery tubes dipped beneath the surface of 30 c.c. of decinormal sulphuric acid contained in 250 c.c. flasks provided with a mark at 180 c.c. volume. In one of the pyrex reaction flasks, which served as the control, was placed 50 c.c. of the extract, water to 240 c.c. and a few pieces of porcelain to promote smooth boiling. 10 c.c. of 25 per cent. sodium hydroxide solution was then added and the stopper carrying the splash-bulb inserted. The other flask had similar contents, but in addition 1 gm. of Devarda's alloy was added. The flasks were warmed by very low gas flames, actual boiling or distillation being avoided until the reaction between the alloy and the alkali had subsided. Heating was then continued more vigorously but always in such a way that both control and determination were distilling at the same rate, and 150 c.c. of distillate collected. The condensers were disconnected and rinsed out and the excess of acid in the two flasks determined by back titration with decinormal sodium hydroxide using methylred as indicator. Duplicate determinations were found to agree excellently and, as already pointed out by Strowd, the method gives results in good agreement with the gasometric method of Schultz. In making the final calculations, the volume of acid neutralized by the control was subtracted from that in the determination since sodium hydroxide alone is capable of liberating ammonia or volatile amines from some of the organic constituents of the plant, e.g. bases like choline, etc.

As an example of the method, the figures obtained in one experiment on Onderstepoort *Tribulus* are reproduced below:—

25 gm. dry plant extracted by 500 c.c. boiling water in two lots of 250 c.c. each. Nitrate determination upon 50 c.c.

	c.c.
(i) Acid neutralized in blank determination	1·8
Acid neutralized in nitrate determination	10·7
Therefore due to nitrate	8·9

Since the acid was 0·09523 normal this is equivalent to 8·9 × 0·09523 × 101 mgm. KNO_3 or 3·42 gm. KNO_3 per 100 gm. plant.

(ii) Acid neutralized in blank determination	1·4
Acid neutralized in nitrate determination	10·1
Therefore due to nitrate	8·7

i.e. 3·35 gm. KNO_3 per 100 gm. plant.
Mean of determinations 3·39 gm. per cent.

The above figure is typical of that found by this method in many analyses of dried Onderstepoort *Tribulus*. The average figures for the other two varieties are given below for comparison.

Nitrate content of *Tribulus* samples (dried material):—

Onderstepoort *Tribulus*: 3·39 gm. KNO_3 per 100 gm.
Northern Transvaal: 2·29 gm. KNO_3 per 100 gm.
Melton Wold *Tribulus*: 1·20 gm. KNO_3 per 100 gm.

It is clear that these plants are considerably richer in nitrate than are most dicotyledons. It is also apparent that the nitrate content is more than sufficient to account for the quantities of nitrite found experimentally and demanded by the minimal quantity of the plant juice or extract known to be toxic when fed to sheep.

Although the Devarda alloy method has been shown by Strowd to be reliable it was felt that confirmation of these figures by some other method would be desirable in view of the importance to be attached to them in the elucidation of the whole problem of the toxicity of this plant.

At the time of conducting the experiments the method of Pucher, Vickery and Wakeman (1932) had not yet reached us, however, the very specific colorimetric phenol-sulphonate method used in the determination of nitrates in water analysis was adopted and found to give results in excellent agreement with those yielded by the reduction method.

The phenol-sulphonic acid reagent was made up according to Charnot, Pratt and Redfield (1911) by dissolving 25 gm. phenol in 150 c.c. pure concentrated sulphuric acid and adding 75 c.c. of fuming sulphuric acid (13 per cent. SO_3) mixing and heating in a boiling water bath for 2 hours.

Since the method is capable of determining 10 parts of nitrate nitrogen per million, an extensive dilution of the plant extract could be carried out thereby very considerably diminishing the quantities of chloride and organic matter present. The following example will serve to illustrate the procedure and to demonstrate the agreement between the two methods.

An extract was made in the usual way of 20 gm. of *Tribulus* by 200 c.c. of boiling water. Nitrate determination by the Devarda alloy method gave values corresponding to 2·74 and 2·86 mgm. per c.c. or 2·74 per cent. and 2·86 per cent. KNO_3 respectively in the original plant powder. Mean 2·80 per cent.

1 c.c. of the filtered extract was diluted to 100 c.c. and of this solution duplicate samples of 10 c.c. each were mixed in centrifuge tubes with 5 c.c. of a suspension of freshly precipitated aluminium hydroxide. After centrifugation, 10 c.c. of each of the supernatant liquids plus 2 drops of decinormal sodium hydroxide were pipetted into small evaporating basins. A range of standards were similarly prepared by taking suitable quantities of a standard potassium nitrate solution. All solutions were evaporated to dryness upon the water bath, 2 c.c. of the phenol-sulphonic acid reagent was then added and after thorough mixing of this with the residue, 20 c.c. of water was carefully added to each (it was found convenient to float the dishes in a large basin of water in order to avoid great development of heat in this and the next stage). After stirring, 10 c.c. of a 10 N solution of potassium hydroxide was then added, which quantity of alkali is slightly more than sufficient to neutralize the acid present, and the resultant yellow solutions compared in vessels of similar size and shape. The dilution of the standard most nearly matching the unknown solutions was picked out and from this the original

nitrate content of the plant extract calculated. In the above case it was found to be 2·70 mgm. KNO_3 per c.c. corresponding to 2·70 per cent. of KNO_3 in the plant powder.

In a later experiment using freshly gathered green *Tribulus* values of 0·577 per cent. and 0·570 per cent. KNO_3 were found by the Devarda method and 0·58 per cent. by the colorimetric method (percentages calculated upon fresh wt.). There is no doubt therefore that in the present case the results of the Devarda method can be relied upon to give the true nitrate contents.

The presence of such a quantity of nitrate in the plant suggested that it would be possible to isolate the salt in the pure crystalline form. This was accomplished by making a water extract, clearing with a considerable quantity of decolorizing charcoal (free from nitrate) and evaporating to a syrup. 96 per cent. alcohol was then added and this mixture filtered. The residue was well washed with 96 per cent. alcohol and then subjected to fractional crystallization. Potassium nitrate was obtained in fair yield.

It would thus appear that the greater part of the nitrate is present in the plant as the potassium salt.

That the production of nitrite from nitrate is brought about by an enzymic reducing system present in the plant is fully capable of explaining the salient characteristics summarised below of nitrate formation in *Tribulus* juice or extracts. What substance acts as the hydrogen donator in the system it is impossible to state:—

1. Absence of nitrite in fresh plant but slow liberation when macerated with water.
2. Thermolability of the system.
3. Abundance of nitrate in the original material.
4. Capability of thoroughly washed plant powder to bring about the reduction of added nitrates.
5. The persistence of the reaction under conditions excluding the possibility of bacterial action.

With regard to findings 4 and 5, the evidence may be presented as follows.

In order to ascertain whether aqueous extracts of the plant powder possessed the reducing activity and could thus be utilized for closer study of the system, 10 gm. of dried Onderstepoort *Tribulus* was allowed to stand overnight in 50 c.c. of 5 per cent. disodium-hydrogen phosphate solution (pH 8·4) to which a few drops of chloroform were added. The liquid was expressed, centrifuged, saturated with ammonium sulphate and the resulting precipitate centrifuged off and washed by saturated ammonium sulphate solution. It was then redissolved in 5 c.c. of water.

This solution was tested for activity by addition of potassium nitrate (25 c.c. of a 0·2 per cent. solution), 3 mgm. of xanthine freshly dissolved in 3 c.c. of N/10 sodium hydroxide and 0·1 c.c. of chloroform. After 4 days the mixture was examined quantitatively for nitrite by means of the Griess-Ilosvay reagent and 0·21 mg. found to be present, i.e. less than 1 per cent. of the amount theoretically derivable from the nitrate added.

5 gm. of the plant residue was freed from nitrate and nitrite by steeping once more in water overnight, squeezing off and then thoroughly washing the residue in repeated changes of distilled water. One quarter of the material so obtained, 2·5 gm. dry wt., was suspended in 50 c.c. of water plus 0·1 c.c. chloroform. An equal quantity was added to 20 c.c. of 0·2 per cent. potassium nitrate solution, 30 c.c. of water and 0·1 c.c. of chloroform added and the two mixtures allowed to stand in stoppered flasks for 4 days at the temperature of the laboratory after which time they were tested for nitrite with the following result, from which it is evident that the nitrate reducing system is retained in the plant material and cannot be washed out by water.

1. Washed residue plus water; Nitrite nil.
2. Washed residue plus KNO_3 solution; 25·36 mgm. i.e. 63·4 per cent. of that theoretically possible.

The remaining 5 gm. of washed residue was used in attempts to reconstitute the system, replacing nitrate by methylene blue (0·5 c.c. of 1 in 5,000), and working at temperatures ranging from 20° to 65° in vacuum tubes of the usual Thunberg type. In some cases xanthine or acetaldehyde was also added but in no instance was any appreciable rate of discoloration of the methylene blue recorded although the pH was also varied over a wide range.

These observations are in agreement with the findings of others who have worked upon plant oxide-reductase systems. Thus Abelous and Alloy (1904) found that, in the presence of salicylaldehyde, potato juice reduced nitrates to nitrites. Bach (1913) interprets this action as being due to a water-splitting oxido-reductase and capable therefore of carrying out the same reaction in the absence of oxygen. The presence of an aldehyde was shown to be essential to complete the system with freshly prepared extracts, although in extracts which have been allowed to stand, its place can be taken by some substance formed as the result of autolysis. Michlin (1928) in a very thorough investigation of the nitrate-reducing system of the potato, showed that this differs in certain fundamental respects from the nitrate-reducing xanthine-oxidase system of milk, the so-called 'Schardinger enzyme.' In the first place, the milk system is destroyed at reactions greater than pH 9·5 whilst that of potato juice is unaffected. In the former, methylene blue can be substituted for aldehyde without diminution of the activity of the system which moreover possesses a wide pH range extending from pH 3·0 to 8·6, whilst in the latter the reductase is practically inactive with methylene blue, even this feeble action, which Bernheim (1928) has shown to be limited to a narrow range between pH 7·3 and 7·8 and to require strict anaerobiosis, being inhibited by addition of m/300 potassium cyanide. The milk enzyme is unaffected in presence of m/50 potassium cyanide. Michlin found that the potato extracts with which he worked had no effect whatever upon the purine bases and that the feeble anaerobic activity with methlylene blue is only shown by extracts which have been cleared with charcoal. He suggests that the difference between the plant and animal systems may be due to the absence of some co-enzyme or carrier from the former.

In the present work the best antiseptics for use with the *Tribulus* system were found to be toluol, chloroform (0·2 per cent.) or ether (0·4 per cent). Formaldehyde (0·2 per cent.) exerted a very pronounced inhibiting action which is in accordance with the experience of Bach (1911) who found both the Shardinger enzyme and the oxido-reductase of liver to be markedly inhibited by formaldehyde or even by acetaldehyde.

DETERMINATION OF NITRATE CONTENT IN FRESH PLANT MATERIAL.

Since it appeared likely that the existence in *Tribulus* of an energetic reducing system would lead to decomposition of nitrate during the drying process, efforts were made to determine the quantity of nitrate present in the freshly gathered green plants.

For this purpose, two sources of supply were used: plants raised from the seed of local *Tribulus* and growing in the Onderstepoort poison garden, and *Tribulus* which was found growing next to one of the sheep camps in the laboratory yard and which had evidently seeded itself from material used in feeding experiments.

Determination of nitrate content was carried out as follows. A 50 gm. to 100 gm. sample was dropped into ten times the volume of boiling water, ebullition maintained for two or three minutes, a few c.c. of toluol added and the flask closed and allowed to stand until next day. After straining off the liquid and squeezing, the residue was put through a small mincing machine and returned to the flask with one half or an equal quantity of water to that first used. All parts of the mincer and all vessels used were rinsed in this operation.

The contents of the flask was brought to boiling and extraction allowed to take place as before. The two extracts were combined and their volume measured, aliquot portions of about 200 c.c. being taken for nitrate determination by the Devarda alloy method, or after suitable dilution, for the phenol-sulphonic acid colorimetric procedure. Results were checked by duplicate determinations. A representative sample of the fresh material was always taken for moisture determination, drying being continued at 95° to 100° until the weight remained constant.

Most surprising differences in nitrate content were found both between plants gathered on different days (after rain, etc.), and even between specimens taken at the same time from the same small plot and differing only in their outward appearance and condition of growth—luxuriant or stunted. That the technique was not at fault, nor errors of sampling too large, was proved by the agreement of duplicates. In the table immediately following, some of the more striking results are reproduced. Nitrites were never found to be present in quantities exceeding the merest traces in any of the specimens tested.

TABLE

Variation in Nitrate Content of Freshly Gathered Tribulus.

Source of Material.	Date of gathering.	Description.	Moisture content	KNO_3 content per cent. of wet weight.	KNO_3 content per cent. of dry weight.
Laboratory poison garden	9.30 a.m. of 28/10/32	Well grown	76·6	50 gm. sample 0·58 (colorimetric 0·58) 30 gm. sample 0·57	2·44
” ”	2 p.m. of 2/11/32*	Well grown	70·6	100 gm. sample 0·39	1·32
” ”	10 a.m. of 7/11/32	Luxuriant,	74·0	50 gm. sample 0·40	1·53
		Ditto, after drying			1·33
” ”	”	Stunted, poorly grown	74·0	50 gm. sample 0·03	0·12
Laboratory yard, sheep camp 47	Noon, 7/11/32	Well grown,	74·4	50 gm. sample 1·04	4·06
		Ditto, after drying			2·96
Laboratory poison garden	10 a.m., 5/1/33	Single large plant in plot, very well grown	82·17	50 gm. sample 1·27	7·12
Bethulie, O.F.S......	10/12/32	Small plants	—	20 gm. sample (dry)	0·65
Middelburg, C.P.....	8/2/33	Stunted: Gathered at scene of outbreak of dikkop	—	10 gm. sample (dry)	1·42

* Fairly heavy rain fell between 28/10/32 and 2/11/32.

The very great variability is apparent of the nitrate content found although in all of the above cases only one species of *Tribulus* was being considered. The very high nitrate content of the plants growing near to the sheep camp is probably to be explained by the rich manuring of such soil. With regard to the other variations, especially the difference between the luxurious and the stunted plants from the same plot little can be said by way of attempted explanation.

That the amount of nitrate present in a plant may vary very considerably was pointed out so long ago as 1884 by Berthelot (1884). Anderson (1924) has recently reported that *Mercurialis perennis* also shows a seasonal variation, the test for nitrate being positive in October but negative in June. The leaves of *Lupinus sp.* follow a similar cycle, whilst in the case of *Solanum Dulcamara L.* the nitrate content was much higher in the early morning than in the evening. Locality also influences the nitrate content very markedly as is shown by the examples of *Suaeda fruticosa*, free from nitrate when growing on highly insolated shingle, but giving a strongly positive test when grown in shaded garden soil, and *Sambucus nigra* which was very rich in nitrate when growing in shaded valleys.

Klein (1913) has shown that the distribution of nitrate in the different parts of the plant varies largely.

The activity of the nitrate-reducing mechanism according to Anderson (1924) also fluctuates considerably. *Solanum Dulcamara*, for example, exhibits a very high activity in June, the season at which the nitrate content of this plant was at its lowest, but evinces such a feeble activity in October as to be considered practically negative.

How the activity of the enzyme system may affect the nitrate content of *Tribulus* plants, as also the effect of growth, locality, etc., it is at present impossible to state with certainty. Such variations may conceivably bear some relationship to the periodic and fluctuating character of geeldikkop.

FEEDING EXPERIMENTS AND ANIMAL TESTS.

Experiment 1.—Feeding Green Tribulus plants to Sheep at Onderstepoort.

As pointed out previously (15th Report, D.V.S. p. 765), cases of geeldikkop were produced in sheep in the Burghersdorp district in January, 1929, when animals were fed exclusively on green *Tribulus* plants.

On account of rain falling while the experiment was in progress, the disease disappeared abruptly, so that the work had to be discontinued. However, in February, 1932, a similar experiment was started at Onderstepoort, where a small patch of the locally found *Tribulus* (*Tribulus murex*?) was established. When in the flowering stage, and growing luxuriantly, the plants were allowed to be grazed for one hour twice daily by a merino lamb about 6 months old, not receiving any other food besides the *Tribulus*. The animal was exposed to sunlight for several hours each day. After seven days feeding on *Tribulus* the sheep started losing condition, and after three weeks it was very weak and emaciated, although throughout the whole period *Tribulus* was readily being eaten. At no time did the animal become photosensitive, or did any swellings or icterus develop. Blood samples were drawn each day and the van den Bergh test carried out. This remained negative.

After three weeks of grazing on the plot, it was decided to dose the juice from 2 kilos of the same fresh plant. Within three hours after dosing the animal died of acute methaemoglobinaemia. Except for the intense chocolate brown colour of the blood no other lesions were to be found.

Although from the above single experiment no conclusions can be drawn, as to the ability of this plot of *Tribulus* to produce true cases of geeldikkop, seeing that similar experiments carried out in an affected area may fail, it nevertheless clearly shows that the juice even from a comparatively small amount of the green plant can be very toxic, although the immediate cause of death in this case is different (methaemoglobinaemia) from that encountered in sheep dying from geeldikkop under natural conditions (icterus, oedema and skin necrosis).

Experiment 2.—Repeated drenching with dried Melton Wold Tribulus

This *Tribulus* was collected on the Melton Wold Estate in the Victoria West district during an outbreak of geeldikkop on the estate. The material was air-dried, finely powdered up and dosed to sheep at Onderstepoort.

One sheep was dosed daily for 10 days with 500 grams of the powder freshly mixed in 3 litres water, and the animal kept in the sun. No signs of illness were noted during this time.

Unless specifically stated, all extractions were made by allowing the dried plant material to macerate in water for several hours, usually overnight, under conditions which permitted any enzymic changes to take place.

One sheep dosed with the watery extract from 1 kilo of the same dried Melton Wold *Tribulus*, died from acute methaemoglobinaemia within three hours.

It is thus clear that dry Melton Wold *Tribulus*, as is the case with Onderstepoort *Tribulus* does not produce true geeldikkop when dosed repeatedly, although acute methaemoglobinaemia again is the constant cause of death, when the fermented watery extract is dosed.

Experiment 3.—Drenching of Tribulus *Extracts.*

In this experiment 31 young merino sheep were used. The routine procedure was to dose all sheep through a stomach tube. All animals were closely shorn and kept exposed to sunlight between hurdles. Any shade was avoided as far as possible. The object of the experiment was to ascertain whether regular dosing of different extract of *Tribulus* plants would ultimately lead to the typical disease. In addition to the dose a little roughage and green lucerne were also allowed, unless otherwise stated.

One sheep was dosed with freshly expressed juice from 1·5 kilos green Onderstepoort *Tribulus*. Although the animal became dull and listless within a few hours after dosing, it was apparently completely recovered the next morning, when the same amount of juice was again dosed. The animal died in the afternoon from intense methaemoglobinaemia.

From this experiment it may be concluded that death was due either to the cumulative effect of the toxin in the two doses, or only that the body had been rendered more susceptible by the first dose, which in itself produced only very slight symptoms.

It may be pointed out that in previous experiments with *Tribulus* juice the minimal lethal dose was found to vary within fairly wide limits, depending apparently not only on the toxicity of the juice, but also on the age and condition of the animal.

Experiment 4.—Drenching with Dried Juice Expressed from Onderstepoort Tribulus.

Ninety grams of dried juice obtained from 2 kilos fresh plant were dissolved in 2 litres water and dosed to a sheep. Death from methaemoglobinaemia followed within 8 hours.

This indicates that desiccation did not in any way effect the toxicity of the juice.

Experiment 5.—Drenching of Watery Extract of Dry Onderstepoort Tribulus.

Some of the green *Tribulus* was air-dried and subsequently finely powdered. Of this 1 kilo amounts were used in these experiments. Four litres of tap water were added to each one kilo of powder and the material allowed to extract overnight. Fermentative changes were generally visible the next morning and a peculiar musty odour was found to have set in. The watery fraction was thoroughly removed by means of a strong, hand-operated press. It was turbid and greenish-brown in colour. As a rule two-thirds to three-quarters of the water added was again recovered in the extract. On dosing the extract so prepared from 1 kilo amounts, sheep were regularly found to die from methaemoglobinaemia in 2 to 6 hours.

Experiment 6.—Drenching of Watery Extract of Onderstepoort Tribulus *in Acid Medium.*

The object of this experiment was to ascertain whether the pH of the extract, or of the rumen of the animal, would effect the toxicity. For this purpose a sheep was dosed on two consecutive days with 500 c.c. of $^{N}/_{10}$ hydrochloric acid. On the third day the animal received the watery extract from 1 kilo of dried Onderstepoort *Tribulus*, to which 40 c.c. concentrated HCl was added just before dosing. No symptoms were noted during the day, but the aimal died during the night, with the blood still somewhat brown the next morning, i.e. death was apparently again due to methaemoglobinaemia.

Another sheep, which was dosed with the watery extract from 1 kilo *tribulus* to which 15 c.c. concentrated HCl was added before dosing, showed respiratory distress within 5 hours, and died shortly afterwards from methaemoglobinaemia.

A third sheep was dosed with the acidified watery extract from 1 kilo dried *Tribulus*. In this case 20 c.c. concentrated HCl was added to 3 litres of water and the *Tribulus* soaked in this overnight. The animal died from typical methaemoglobinaemia within 3 hours.

It may thus be concluded that an acid medium whether of the plant extract, or in the rumen of the animal does not in any way effect the toxicity.

Experiment 7.—Drenching of Watery Extract of Onderstepoort Tribulus *in Alkaline Medium.*

One sheep was dosed with the watery extract from 1 kilo dried Onderstepoort *Tribulus* to which 35 grams sodium carbonate was added just before dosing. The animal died during the night from methaemoglobinaemia.

Another sheep was dosed with an alkaline extract prepared by soaking 1 kilo *Tribulus* in 3 litres water containing 40 grms. sodium carbonate. The animal died within 3 hours, the blood again showing the typical brown discoloration.

From this experiment it is clear that an alkaline medium does not effect the toxicity of the plant extracts.

Experiment 8.—Administration of Sodium Nitrite to Sheep.

Once it was known that a nitrite present in the *Tribulus* extracts was causing the death of the sheep, it was decided to obtain more information about the action of inorganic nitrites when administered in different ways, and it was hoped that by these means light would be thrown on the cause of the symptoms in true geeldikkop.

For this experiment 18 young merino sheep were used. The animals were again closely shorn and kept out in the sunlight each day as in the previous experiments.

(*a*) *Drenching of Sodium Nitrite.*

Eight young sheep were drenched with amounts of sodium nitrite ranging from 1·5 to 2·5 grams per dose. The material was dissolved in a few c.cs. of water and dosed through the stomach tube.

As a rule no symptoms were shown within the first two hours after dosing although a progressive brown discoloration of the conjunctivae could frequently be noted. Soon thereafter a varying degree of respiratory distress was shown. In single doses of 2 grams and more, death usually supervened after the third or fourth hour, the main symptoms being those of acute respiratory distress and asphyxia. In doses of less than 2 grams, most of the animals after a short transitory period of distress, rapidly recovered to apparently normal health. In several cases it was observed that recovery could rapidly take place even after the animal had passed into a state of coma, the brown colour of the blood and mucous membranes soon changing back to the normal red. Where death supervened, the only post-mortem finding was that of an intense methaemoglobinaemia. No signs of haemolysis were ever seen, the serum always remaining perfectly clear and colourless. In these sheep it could therefore be taken that a single dose of 2 grams sodium nitrite represented the minimal lethal dose.

After this, it was decided to ascertain the effects of repeated dosing of sublethal amounts of sodium nitrite. In the case of one sheep, daily dosing was commenced with 1 gram sodium nitrite. On the 9th day this was increased to 1·5 grams, on the 12th day to 2 grams, from the 18th to 2·5 grams and from the 24th day to 3 grams. On the 29th day the animal was discharged after having received 57·5 grams sodium nitrite. Although slight browning of the conjunctivae was noted at different times, no outward symptoms of illness were ever shown. Another sheep was dosed over a period of 41 days with 82 grams sodium nitrite (maximum dose 3 grams), without showing any signs of illness. By the end of this period the red cell volume had increased to 40 per cent. from an initial 26 per cent.

From these experiments it was evident that sodium nitrite alone did not produce any symptoms of true geeldikkop. On the contrary, it became clear that the animals developed a definite tolerance to nitrite and that the volume of red cells was markedly increased. This may have been due to an attempt on the part of the body to compensate for the chronic anoxaemia caused by repeatly dosing with nitrite.

(b) *Drenching of Sodium Nitrite and Potassium Permanganate.*

It was hoped that potassium permanganate when dosed simultaneously with the nitrite would inhibit the toxic effects of the latter. Doses of 0·25 grams potassium permanganate were given daily immediately after the nitrite. In this way one sheep was dosed with 71·8 grams sodium nitrite and 6·5 grams potassium permanganate over a period of 30 days (maximum dose of nitrite 3·5 grams). Although browning of the conjunctivae was noted off and on, no signs of illness were shown until on the 30th day, when the animal developed marked respiratory distress and died of typical acute methaemoglobinaemia, which also was the only post-mortem finding.

(c) *Drenching of Sodium Nitrite with Sodium Bicarbonate.*

One sheep was dosed with 53·5 grams sodium nitrite (maximum amount 3 grams) and 650 grams sodium bicarbonate (maximum amount 50 grams) over a period of 47 days. The animal remained in good health and was discharged on the 50th day, indicating that the sodium bicarbonate had in no way influenced the course of events.

(d) *Intravenous Injections of Sodium Nitrite.*

Injected intravenously 0·75 to 1 gram sodium nitrite was regularly found to cause death of sheep from methaemoglobinaemia within a few hours. Repeated daily injections starting with 0·1 gram and gradually increasing to 0·8 gram usually produced no symptoms. With doses above 1 gram animals may, however succumb very rapidly. In the case of a dog, 0·5 grams intravenously caused death from methaemoglobinaemia within 2 hours, while another dog of the same size withstood 11·5 grams sodium nitrite injected over a period of 23 days. One sheep injected intravenously with a total of 10·8 grams (maximum dose 1 gram) died after the 23rd day with acute brown discoloration of the blood.

(e) *Influence of Starving on Sodium Nitrite Poisoning.*

One sheep was starved for 48 hours and then dosed 2 grams sodium nitrite. Death took place within 2 hours, the blood being intensely brown.

Another sheep similiarly starved was dosed 5 oz. meatmeal together with 2 grams sodium nitrite. Death followed within 6 hours, the blood again being intensely brown.

(f) *Drenching of Sodium Nitrite followed by Injection of Haematoporphyrin.*

One sheep was dosed 2 grams sodium nitrite on two consecutive days, after which 0·2 gram haematoporphyrin was injected intravenously. Photosensitisation became very marked soon afterwards. A subsequent dose of 2·5 grams sodium nitrite rapidly caused death from methaemoglobinaemia.

Discussion.—From the above experiments it is clear that sodium nitrite is very poisonous to sheep, although as a rule repeated sublethal doses provoke a well-marked tolerance. Death, when it takes place, always results from an intense methaemoglobinaemia. In no case does sodium nitrite poisoning lead to symptoms simulating those seen in true geeldikkop, the clinical picture and post-mortem findings resembling those reported in the literature for other animals. No essential difference was found between sodium nitrite and potassium nitrite poisoning. Higher doses of the latter (about 3·5 to 4 gm.) were required to produce death.

Experiment 9.—*Drenching of Potassium Nitrate.*

Since it had been shown that potassium nitrate as such was contained in the fresh *Tribulus* plant, it was thought advisable to dose some of the pure salt to sheep. One sheep dosed with 10 grams potassium nitrate on two consecutive days died within 6 hours after the second dose from typical methaemoglobinaemia. Another animal only died after receiving 80 grams potassium nitrate (maximum dose 20 grams) over a period of 5 days. One sheep dosed with 75 grams of potassium nitrate over a period of 14 days developed no symptoms.

It was thus shown that large doses of potassium nitrate may cause death in the same way as does the nitrite.

Experiment 10.—*Administration of Hydroxylamine Hydrochloride.*

The object of this experiment was to ascertain whether a product such as hydroxylamine, which has been detected as an intermediate in the biological reduction of nitrates to ammonia (see Blom 1928), showed any toxic effect on sheep. One sheep was dosed with 16·25 grams (maximum dose 1·5 grams) over a period of 21 days without any ill effect. Another sheep injected with 0·5 grams intravenously showed marked shock-like symptoms, but soon recovered. Three subsequent injections of 0·3 gram produced haemolysis which resulted in the death of the animal on the 6th day.

Experiment 11.—*Administration of Hydrazin Sulphate.*

One sheep was injected intravenously with 0·3 grams hydrazin sulphate daily for 11 days without showing symptoms. Thereafter it received two injections of 0·6 grams each. This produced a transitory weakness lasting two or three days. The animal was then dosed with 1 gram hydrazin sulphate daily for 8 days. The only symptom noticeable was a progressive weakness in the legs, which soon passed off after dosing had been stopped.

Experiment 12.—*Drenching of Ammonium Carbonate.*

The object here was to note whether the NH_4 ion would produce symptoms in a sheep. One sheep was dosed with 244 grams (maximum dose 24 grams) of Ammonium carbonate over 24 days. No symptoms whatever were shown, until on the last day the dose was increased from 20 grams to 24 grams. Three hours after receiving the last dose, the animal suddenly became very weak, the whole body trembling markedly, lips were parted, respirations very fast, there was stiffness in the hind limbs, and frothing from the mouth and nostrils. The animal died within 4 hours after dosing. No signs of methaemoglobinaemia were noticeable on post mortem.

Experiment 13.—*Drenching of Potassium Nitrite, Potassium Nitrate, Ammonium Carbonate, Hydrazin Sulphate, and Hydroxylamin Hydrochloride.*

One sheep was dosed with 252 grams ammonium carbonate, 81 grams potassium nitrate, 20·25 grams potassium nitrite, 20·25 grams hydroxylamin hydrochloride, and 15·75 grams hydrazin sulphate over a period of 43 days. The animal became progressively weaker, without showing any other symptoms. Death occurred on the 43rd day, the only post mortem finding being that of cachexia and general atrophy. It was hoped that by simultaneously dosing of these different nitrogenous compounds, characteristic symptoms might possibly be shown by the animal. This however is not the case.

Experiment 14.—*Drenching of Nitrobenzol.*

One sheep was dosed 0·5 c.c. nitrobenzol on two consecutive days without any ill effect. On the third day it received 2 c.c. Within 1 hour the animal was found to be *in extremis*. It died half an hour later from acute methaemoglobinaemia. Another sheep was dosed 0·5 c.c. on five consecutive days without ill effect. On the 6th day 1 c.c. was given. This caused death within one hour. Nitrobenzol was thus found to be extremely toxic to sheep, death being due to very rapid methaemoglobin formation.

Experiment 15.—*Drenching of Nitrobenzaldehyde.*

One sheep was dosed with 29 grams nitrobenzaldehyde (Maximum dose 4 grams) over a period of 20 days without showing any symptoms, except a slight haemolysis, noticed in the blood serum.

Experiment 16.—*Drenching of Amyl Nitrite.*

One sheep was dosed with 49 c.c. amyl nitrite over a period of 24 days without any symptoms. Another sheep, which received 1 c.c. amyl nitrite intravenously died from methaemoglobinaemia within a few hours.

SUMMARY.

1. It has been demonstrated that a lethal factor is present in the fresh juice expressed from green *Tribulus* plants and in the watery extracts prepared by soaking the dried ground plant material in water for several hours.

2. Death, in each instance, is due to acute asphyxial conditions resulting from the rapid intra-corpuscular change of haemoglobin into methaemoglobin.

3. The agent responsible for this change has been identified as inorganic nitrite—chiefly potassium nitrite.

4. Nitrite as such is absent (except in traces) from the tissues of *Tribulus* plants, but is formed from pre-existing nitrate (isolated in crystalline form) under the influence of an enzymic oxidation-reduction system.

5. The properties of this system have been examined and are found to conform in general to those of other plant oxido-reductases, e.g. that of the potato.

6. The production of nitrite by the plant from added potassium nitrate has been demonstrated.

7. Methods are described for the determination of nitrate in fresh green *Tribulus* plants and in the dried ground material.

8. It has been shown that not only does the nitrate content vary widely in different species of *Tribulus* but also in different plants of the same species and growing in the same locality.

9. Prolonged feeding of fresh green or dried *Tribulus* under the Laboratory conditions failed to produce any ill-effect whereas under field conditions, in the Karroo area, as is well known, grazing of this plant may in certain circumstances give rise to outbreaks of geeldikkop, a disease characterised by an intense photosensitisation and accompanying generalized icterus.

10. Administration of sodium and potassium nitrites, nitrobenzol and amylnitrite all resulted in death from simple methaemoglobinaemia.

11. Prolonged administration of graduadly increasing doses of sodium nitrite provoked a well marked tolerance in sheep.

12. Repeated dosing over an extended period of sodium nitrite, hydroxylamine hydrochloride, hydrazine sulphate, and ammonium carbonate, substances regarded as possible intermediates in the biological formation of ammonia from nitrates failed to produce either photosensitivity or icterus.

Work is being continued in an endeavour to throw further light on the problem of geeldikkop.

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Onderstepoort Journal of Veterinary Science and Animal Industry, Volume I, Number 2, 1933.

Studies on the Photosensitisation of Animals in South Africa.

III. The Photodynamic Action of *Hypericum ethiopicum var. glaucescens* Sond. and *Hypericum leucoptychodes* (Syn. *H. lanceolatum* Lam.).

By J. I. QUIN, D.V.Sc., Veterinary Research Officer, Onderstepoort.

INTRODUCTION.

It is a well-known fact that several species of the genus *Hypericum*, when eaten by animals, cause photosensitisation of the unpigmented skin. Thus according to old Italian literature *Hypericum crispum* was regarded as dangerous for sheep, but only so if the animals had unpigmented white skins. Since that time various investigators have published accounts on the so-called "St. John's wort poisoning of animals". According to Marsh and Clawson (1930), who carried out experiments with *Hypericum perforatum*, collected in Northern California, cattle and sheep when fed on it develop a high temperature, rapid pulse and respiration; while the symptoms of photosensitisation were either absent or very mild. The conclusion which they arrived at was that St. John's wort could not be considered a serious stock poison in California.

Although in South Africa, *Hypericum* poisoning in stock has never been reported, it was thought advisable to conduct some experiments on sheep and goats with locally growing species, the object being to ascertain in how far the symptoms of photosensitisation corresponded with those seen in true geeldikkop caused by the Genus *Tribulus*, and possibly other plants. For this purpose two species of *Hypericum* were collected, both from the Transvaal, and feeding and drenching tests carried out on sheep.

A. Experiments with *Hypericum ethiopicum var. glaucescens* Sond. (National Herbarium No. 12953.

The plants were collected with the kind help of Mr. C. A. Smith during the summer (February) on the hill slopes round about Pretoria, and were in the late flowering stage. After allowing to dry, they were finely powdered up. In this form the material suspended in water, was dosed through a stomach tube to young Merino sheep, closely shorn and exposed to strong sunlight. Altogether six sheep were used.

One sheep dosed with 20 gm. in 3 litres water daily for two consecutive days, suddenly died after the second dose with symptoms of hurried respiration and ingesta running from the mouth. Post-mortem examination revealed generalised cyanosis and pulmonary oedema. In this case the dose had obviously been too bulky, so causing asphyxia.

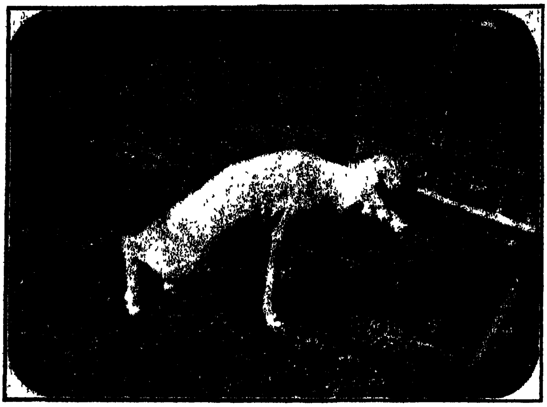

Fig. 1. Acute photosensitisation after dosing *H. ethiopicum.*

Another sheep was dosed with 100 grams powder daily for two days. On the afternoon of the second day, the animal became slightly restless, occasionally rubbing the head against the fence, scratching the ears and licking the lips. On the third day at 10 a.m. it was dosed 250 gm. powder. During the course of the morning, marked symptoms of irritation were shown, scratching of the head, and flinching of the body, causing the animal to assume a crouching attitude (see figs. 1 and 2). The sun seemed to strike the animal along the spine and especially over the croup, and causing it to lie down and rise at frequent intervals. Later in the afternoon, swelling of the ears was clearly evident. The temperature rose from 101·2° F. in the morning to 105·6° F. in the afternoon, while the respirations became markedly accelerated. The animal was not dosed again. On the fourth day since starting the experiment, the ears were markedly swollen, while the other symptoms remained unabated. On the 9th day the swelling of the ears started to subside, although photosensitisation was clearly shown up to the 15th day, the symptoms appearing as soon as the animal was placed out in the sun. From the 16th day onwards there was a progressive hardening of the skin all along the back where the wool had been clipped short. When this part was touched the animal showed marked flinching. The appetite and general health otherwise was good. By the end of a month large flakes of dried skin and wool could be removed from the back (see fig. 3). The temperature curve became normal from the 5th day onwards. Blood from the jugular vein was collected daily up to the 18th day, and after centrifuging, the serum was examined for bile pigments. At no time could these be demonstrated, the serum remaining practically water clear, neither were any clinical signs of icterus visible.

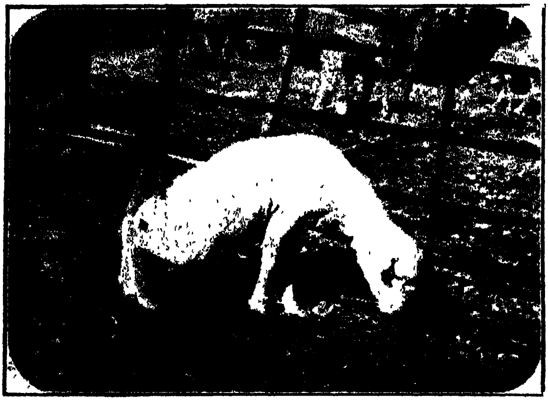

Fig. 2. Acute photosensitisation after dosing *H. ethiopicum*.

Another sheep was dosed 250 gm. powder. The following day the animal became restless and the ears started swelling. On the 3rd day photosensitisation was extreme and the body thrown into an abnormal crouching position. On the 5th day the sheep was only slightly sensitive. On the 6th day it was again dosed 250 gm. powder. Although this caused the animal to go off its food for a few days, no signs of sensitisation were again shown. The serum, as in the previous case, remained water clear.

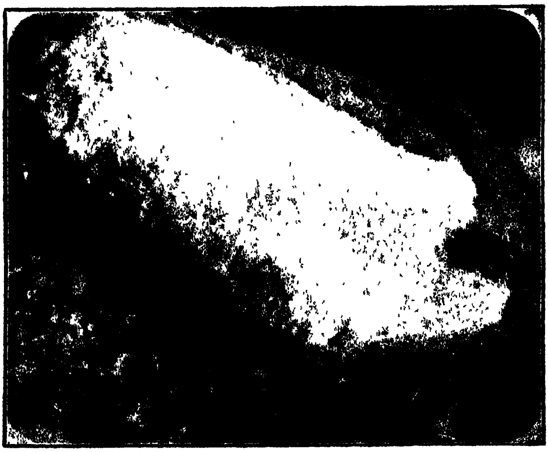

Fig. 3. Chronic skin lesions after repeated dosing of *H. ethiopicum*.

EXTRACTION OF FLUORESCENT PIGMENT FROM THE DRY POWDERED PLANT.

According to Cerny, a deep red fluorescent pigment, which he named hypericin, is contained in various *Hypericum* species, while Hausmann and Zaribnicky have shown that alcoholic extracts prepared from the flowers of *Hypericum perforatum* possessed a sensitising action on erythrocytes.

Extracts prepared by soaking the dry powdered *Hypericum ethiopicum* in acetone overnight, showed a striking deep red fluorescence. Spectroscopically two bands closely resembling those of oxyhaemoglobin were seen. On evaporation of the extracts a deep red slightly sticky residue was left behind. Of this residue 0·5 gm. dissolved in 20 c.c. distilled water and then filtered, was injected intravenously into a sheep. Within a few minutes after injection the animal started showing signs of sensitisation, shaking and scratching the head. Later in the afternoon diarrhoea set in and the animal was found dead the next morning. There were no visible lesions in any of the organs except a catarrh of the intestines.

DISCUSSION.

From experiments carried out with *Hypericum ethiopicum* drenched to sheep, it is clearly evident that this species, as is the case with several other species, contains a strong photosensitising principle capable of producing marked symptoms in sheep with unpigmented skins. When exposed to sunlight such animals exhibit irritation of the exposed parts to such extent, that the body is thrown into various abnormal attitudes, the animal continually seeking shade. There is marked swelling of the face and ears, followed in chronic cases by sloughing of the affected skin. Symptoms of icterus (clinical or blood) are not to be seen.

The plant contains a deep red fluorescent pigment soluble in alcohol, acetone and water. Crude extracts show absorption bands as follows: aqueous, 560 and 600A, acid ether 540, 568, 582; Amylalcohol 528, 540, 570, 583, and probably identical or closely related to that described by Cerny for other *Hypericum* species. This pigment causes photosensitisation when injected into sheep, with symptoms closely resembling those produced by injections of haematoporphyrin (Quin).

B. Experiments with *Hypericum Leucoptychodes* (Syn. *H. lanceolatum* Lam.) (Nat. Herbarium No. 11416).

Through the courtesy of Dr. E. P. Phillips, Division of Plant Industry, a good amount of the above plant was collected at Broederstroom, Northern Transvaal, towards the end of the summer. It was much taller than *H. ethiopicum* and also in the late flowering stage. As in the previous experiments the material was dried and finely powdered before being dosed to animals. One Angora goat and three Merino sheep were used. All the animals were closely shorn and exposed in the sun after dosing.

One Angora goat received 3.250 gm. of the powder in a period of 17 days. On the 10th day it started showing slight photosensitisation, which, however, passed off the following day. The animal, however, became progressively more dull each day, and the blood serum reached a fairly yellow tint. From the 18th day the animal started to purge severely and the dosing was stopped. Death took place on the 22nd day with marked exhaustion following on the severe purging. On post-mortem examination there was a marked catarrhal enteritis.

One Merino sheep dosed 200 gm. powder daily for three days, developed a progressive dullness lasting up to the 11th day, when recovery seemed complete. There were no signs of photosensitisation. The blood serum was definitely yellow from the 3rd to the 10th day.

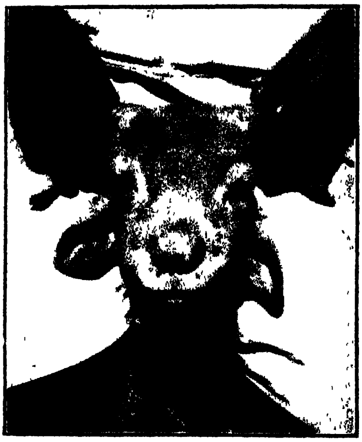

Fig. 4. Swelling of face and ears after dosing *H. leucoptychodes*.

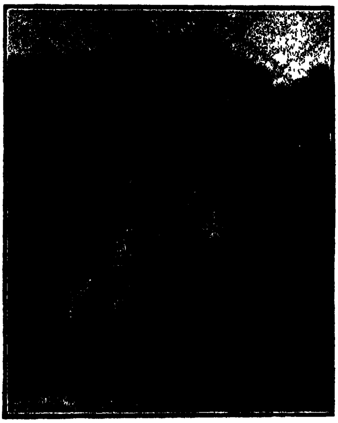

Fig. 5. Swelling of face and ears after dosing *H. leucoptychodes*.

Another sheep dosed 150 gm. daily for 5 days suddenly died during the night of the 5th day without having shown any symptoms. On post-mortem the fore-stomachs were found to be markedly distended.

One Merino sheep was dosed 150 gm. daily for 4 days, followed by 200 gm. daily for 6 days. The animal showed no symptoms until the 9th day, when the ears were found to be markedly swollen, although obvious symptoms of irritation were absent. The oedematous swelling increased and also spread to the face (see figs. 4 and 5) and intermandibular space. On the 16th day the swellings were ± completely subsided. The animal, however, became progressively weaker. On the 24th it was in extremis and consequently killed for post-mortem. Except for the marked atrophy of the muscles and poor condition of the carcass generally, no other pathological lesions were found.

DISCUSSION.

Hypericum leucoptychodes, when repeatedly drenched to sheep causes either no photosensitisation or a delayed and slowly progressive sensitisation to sunlight in the form of a well marked oedema of the subcutaneous tissues of the head and ears unaccompanied by the acute symptoms of irritation and flinching as noticed with *Hypericum ethiopicum*. It does, however, exert a toxic effect on the animal as shown by the severe purging and the progressive debility and inanition.

SUMMARY.

1. Dried pulverised *Hypericum ethiopicum* when drenched to Merino sheep was found to cause intense photosensitisation and oedematous swellings of the exposed parts of the skin. Such affected skin later became necrosed and was followed by sloughing.

2. A deep red fluorescent pigment, soluble in acetone, alcohol and water has been extracted from the dried plant.

3. This pigment when injected into sheep causes well marked photosensitisation.

4. The symptoms are not accompanied by icterus and resemble those produced by haematoporphyrin.

5. *Hypericum leucoptychodes* causes a much weaker and delayed photosensitisation, although diarrhoea is frequently noticed after repeated dosing of the plant to sheep.

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Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 1, Number 2, 1933.

Studies on the Photosensitisation of Animals in South Africa.

IV. The Toxicity of *Lopholaena coriifolia* (Harv.) Phill. & C.A. Sm. (= *L. randii* sp. Moore).

By J. I. QUIN, D.V.Sc., Veterinary Research Officer,
Onderstepoort, Pretoria.

INTRODUCTION.

INVESTIGATIONS into an outbreak of "geeldikkop" amongst sheep on a farm near De Aar, Cape Province, revealed the interesting fact that practically no *Tribulus* plants were to be found on the veld where the animals had taken ill. As it had been shown experimentally that in the Karroo area, wilted *Tribulus* frequently caused the disease during the summer months, this outbreak near De Aar necessitated the investigation of other possible factors in the etiology of the condition. From the symptoms and post-mortem lesions shown by the affected sheep, the disease was diagnosed as true geeldikkop as caused by *Tribulus*.

According to the owner and the shepherds on the property, young sheep were frequently seen nibbling at the soft tops of a plant called "Vaalbos". Some of the plant in the late flowering stage was collected and subsequently identified as *Lopholaena coriifolia* (Harv.). Feeding experiments were then commenced at Onderstepoort with plant material forwarded at regular intervals by Government Veterinary Officer Keppel, De Aar.

FEEDING EXPERIMENTS.

As stabled sheep consistently refused to ingest any of the fresh plant offered to them, it was decided to dry the material, then pulverise and drench through a stomach tube. The plant contained a large amount of tough woody stems and twigs and even the small leaves were fairly leathery. The dry material after being pulverised in a mill emitted a peculiar resinous odour.

Altogether 17 young Merino sheep were used in these experiments. Before being dosed the animals were closely shorn and thereafter kept exposed in sunlight, in order to notice whether symptoms of photosensitisation developed. The following table indicates the results obtained from dosing with the powdered *Lopholaena* plant:—

Sheep No.	Period of Dosing.	Total Amount Dosed.	Symptons Shown.	Post-mortem Findings.	General Remarks.
19435	2 days	400 gm.	Second day: Restlessness and respiratory distress. Third day: Accelerated respiration, listlessness, off feed, abdomen distended. Died fourth day.	Pulmonary congestion, degeneration of myocard. Severe fatty changes in liver.	No icterus or oedematous swellings.
19468	3 days	400 gm.	Second day: Listlessness, jerky intermittent breathing resembling Cheyne-Stokes. Third day: Breathing unchanged. Fourth day: Animal dead.	Severe fatty changes in the liver.	No icterus or oedematous swellings.
21473	15 days	1250 gm.	Sixteenth day: Progressive falling off in condition, breathing hurried and intermittent, drowsiness, lips and conjunctivae light yellow. Animal killed for post-mortem.	Striking fatty changes in the liver	Slight icterus. No oedematous swellings.
19433	14 days	900 gm.	Twelfth day: Listlessness, unsteady gait, falling off in condition, lips yellowish. Fifteenth day: Animal killed for post-mortem.	Slight generalised icterus. Extensive fatty changes in the liver.	Slight icterus, no swellings. Direct v.d. B. test on serum positive.
21461	10 days	1300 gm.	Ninth day: Facial skin slightly yellowish. Eleventh day: Animal killed for post-mortem.	Slight generalised icterus. Fatty changes in liver and kidneys.	Slight icterus, No swellings.
20902	2 days	Aqueous extract from 800 gm.	Fourth day: No symptoms shown.	—	Watery extract produced no effect.
22421	2 days	400 gm.	Second day: Listlessness. Third day: Animal dead.	Extensive fatty changes of liver.	No icterus or swellings.
22418	1 day	200 gm.	Second day: Animal ill, not feeding. Died the same day.	Marked fatty changes of liver. Atony of fore-stomachs.	No icterus or swellings. Death very acute.
22425	1 day	70 per cent. alcoholic extract from 400 g.m.	Fourth day: Animal appears ill, marked dyspnoea. Died during the same night.	Pulmonary congestion. Extensive swelling and fatty changes of the liver and kidneys.	No icterus or swellings.
22391	4 days	300 gm.	Fifth day: Animal very ill and groaning. Mucous membranes injected. Sixth day: Died early in the morning.	Fair generalised icterus. Hydropericardium, oedema of lungs. Very severe fatty changes of liver and kidneys, marked stasis in first part of large intestines.	Fair icterus, no swellings.
22406	3 days	300 gm.	Third day: Animal died suddenly during the night.	Marked fatty changes liver, kidneys, and myocard. Fair icterus.	Fair icterus. No swellings.
22393	2 days	200 gm.	Third day: Animal died suddenly during the night.	Marked fatty changes liver, Pulmonary oedema. Slight icterus.	Slight icterus. No swellings.

From the above table it is clear that the powdered *Lopholaena coriifolia* (Harv.) plant when dosed to sheep causes severe illness even in comparatively small doses of 200 gm. (Sheep Nos. 22418 and 22393). Some sheep, however, require far larger quantities before showing any symptoms, e.g. sheep No. 21461 received 1,300 gm. in 10 days. The symptoms noted were, laboured and hurried respiration, general debility and in some cases a slight clinical icterus. In no case was photosensitisation noticed, although the animals were kept exposed in sunlight. On post-mortem examination intense fatty changes of the liver and frequently also of the kidneys, were the most important lesions to be found. In some cases stasis in the large intestines were also seen. Both the clinical and pathological pictures agreed very closely with the results obtained in either chloroform or phosphorus poisoning of sheep (see subsequent paper), where intense fatty changes of the liver may be provoked within 24 hours of administering the poison. It has also been ascertained that the toxic agent in *Lopholaena coriifolia* (Harv.) is insoluble in water but soluble in 70 per cent. cold alcohol which easily extracts it from the plant. The extract has a strong resinous odour.

CONCLUSIONS.

Lopholaena coriifolia (Harv.), which has been suspected of causing geeldikkop in sheep under field conditions, was dried, pulverized, and dosed to sheep at Onderstepoort. Some animals suffered acute poisoning, while others showed much greater resistance. The symptoms shown did not resemble those of true geeldikkop. The severe fatty change in the liver resembles the action of such poisons as phosphorus and chloroform. In true geeldikkop severe bile stasis and pigmentation of the liver with intense icterus of the whole body are the predominant findings. There is, however, comparatively little fatty change in the liver. From these findings it is evident that *Lopholaena coriifolia* (Harv.) contains an active liver poison which, however, in drenching experiments has not led to the onset of geeldikkop in sheep.

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Onderstepoort Journal of Veterinary Science and Animal Industry, Volume I, Number 2, 1933.

Studies on the Photosensitisation of Animals in South Africa.

V. The Toxicity of *Lippia Rehmanni* (Pears) and *Lippia pretoriensis* (Pears).

By J. I. QUIN, D.V.Sc., Veterinary Research Officer, Onderstepoort.

INTRODUCTION.

In an attempt to produce experimental cases of photosensitisation in sheep, especially with the object of throwing further light on the problem of geeldikkop (*Tribulosis*), various plants were collected and drenched to sheep, and amongst which the abovementioned two *Lippia* species were included. Both species were collected round about Pretoria with the kind help of Mr. C. A. Smith. Most of the plants were in the late flowering or fruiting stage. The fresh material was not readily eaten by experimental sheep, probably on account of the strong characteristic verbenaceous odour. Consequently the plants were dried, pulverized and after mixing in water, drenched to sheep through a stomach tube. Young Merino sheep were used in the experiment. They were closely shorn and kept exposed in sunlight.

A. Experiments with *Lippia Rehmanni* (Pears)

The following table indicates the results obtained with the above plant:—

Sheep No.	Period of Dosing.	Total Amount Dosed.	Symptoms Shown.	Post-mortem Findings	General Remarks.
32598	2 days	400 gm.	Third day: Animal ill, jerking of body, frequently lies down. Fifth day: Purging, condition aggravated, marked icterus, high temperature. Eighth day: Sensitive to sunlight, not feeding. Ninth day: Very ill, marked clinical icterus. Died same day.	Intense icterus, pulmonary oedema. Marked pigmentation and degeneration of kidneys and liver, rumen very dry. Catarrhal enteritis with haemorrhages in large bowel.	Photosensitisation slight, icterus intense.
29454	6 days	600 gm.	Third day: Scratching head and licking lips. Fourth day: Marked flinching in sun, very restless (see photos Nos. 1 and 2). Fifth day: Very sensitive, also clinical icterus. Eighth day: Animal purging. very dull. Fourteenth day: Animal dead.	Intense generalised icterus, pulmonary oedema. Bile stasis, pigmentation liver and kidneys, enlargement of the spleen.	Photosensitisation well marked. Icterus intense.
No number	6 days	600 gm.	Second day: Very restless, scratching head. Fifth day: Marked flinching, licking lips, slight clinical icterus. Seventh day: Still sensitive, icterus more marked, condition falling off. Thirteenth day: Animal dead.	Cachexia and general atrophy, intense generalised icterus, pigmentation liver and kidneys. Bile stasis, enlargement spleen, obstruction in in large intestine.	Photosensitisation well marked. Icterus intense.
33001	7 days	600 gm.	Third day: Very sensitive to sunlight, ears swollen. Sixth day: Still very sensitive, yellowish exudate from base of horns. Tenth day: Skin on lips, ears, and back hardening. Twenty-fourth day: Necrosed skin peeling off from head, ears, and back. Animal discharged.	Animal recovered and discharged.	Marked photosensitisation and swelling of head and ears, followed by slouging of affected skin.
33054	3 days	Alcoholic extract from 600 gm.	Fourth day: Definite photosensitisation. Fifth day: Very sensitive, flinching, restless, lying down and rising. Eighth day: Little sensitive, slight swelling of face and ears. Slight icterus of conjunctivae and base of horns. Serum very yellow, strong direct v.d.B. Tenth day: Swelling subsided, sixteenth day animal discharged.	Animal recovered and discharged.	Marked photosensitisation, with swellings of head and ears. Recovered without sloughing of skin.

From the above table it is clear that the powdered *Lippia Rehmanni* (Pears) when dosed to sheep provokes definite photosensitisation even from the second or third day. There is an accompanying clinical icterus which is strongly marked in the blood serum. In some cases there is swelling of the head and ears which may result in the sloughing of the affected skin, and subsequent complete recovery of the animal. This toxic agent present in the powdered plant can be extracted with 75 per cent. alcohol which when dosed to a sheep produces typical symptoms of photosensitisation. In general, the symptoms produced by drenching this plant, are very similar to those noted in cases of geeldikkop, except that in the latter condition the symptoms are more grave, e.g. the skin of the head and ears frequently shows extensive black dry necrosis, accompanied by rupture of the eyeballs and blindness and hardening and cracking

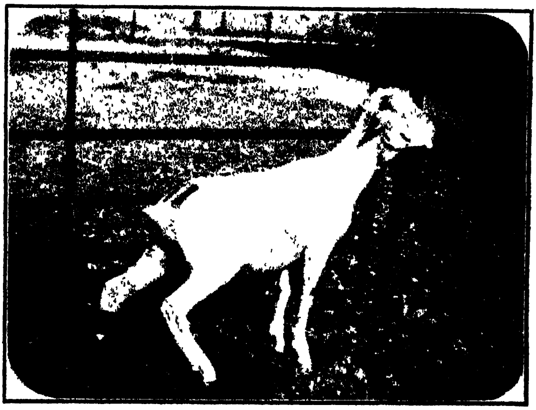

Fig. 1.– Animal flinching in sunlight after being dosed with *Lippia Rehmanni.*

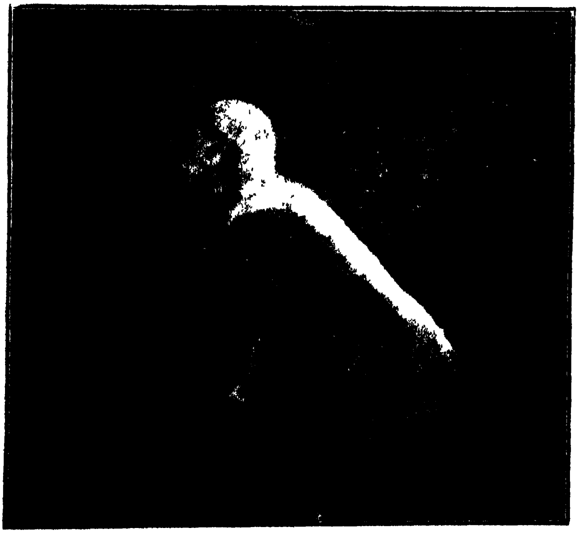

Fig. 2. Animal flinching in sunlight after being dosed with *Lippia Rehmanni.*

of the lips. The icterus, too, is usually far more severe. Thus it would appear that *Lippia Rehmanni* (Pears) contains a toxic principle (perhaps more than one) capable of causing some derangement in the normal function of the liver leading to an obstructive jaundice as shown by a strongly positive direct Van den Bergh reaction of the blood serum. Accompanying this icterus, there is a well-marked photosensitisation with subsequent sloughing of the necrosed skin.

B. Experiments with *Lippia pretoriensis* (Pears) N.H. No. 15175.

This species of *Lippia* was also collected round about Pretoria. It grows in much taller bushes with a large amount of tough woody branches, sparsely covered with small leaves mostly at the top. As in the previous experiments, the material was also dried and then pulverized before drenching to sheep. The following results were obtained:—One Merino sheep was dosed 1,400 gm. dried powder during a period of 14 days. On the third day the animal first showed photosensitisation, e.g. scratching the ears, flinching, pawing the ground and very restless. In spite of continuous daily drenching, these symptoms only lasted four days and then completely disappeared. The animal was discharged on the 15th day in an apparently normal state of health.

Another sheep drenched with 800 gm. powder in 8 days, only showed slight arching of the back on the 6th day, and nothing further.

From these experiments it may thus be concluded that, although the same general type of symptoms are produced by *Lippia pretoriensis* (Pears) it is far less toxic than *Lippia Rehmanni* (Pears), e.g. 1,400 gm. of the first plant caused little effect on a sheep, while 400 gm. of the second species resulted in the death of a sheep on the 9th day.

The symptoms produced by drenching these two species of *Lippia*, although not so marked as those seen in true geeldikkop caused by the genus *Tribulus*, are essentially of the same nature, i.e. photosensitisation accompanied by icterus.

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Studies on the Photosensitisation of Animals in South Africa.

VI. The Effect of Surgical Obstruction of the Normal Bile Flow.

By J. I. QUIN, D.V.Sc., Veterinary Research Officer, Onderstepoort.

INTRODUCTION.

As pointed out in the first of this series of papers, some considerable difficulty is experienced in producing experimental cases of true geeldikkop in sheep. Although several species of *Tribulus* can definitely be held responsible for outbreaks of the disease in the Karroo areas of the Cape Province, artificial feeding of the plants collected and fed to susceptible sheep on the spot frequently yield no positive results. With fresh or dried plant material despatched to the Onderstepoort Laboratory, no single case of the true disease has yet been produced. Similarly, *Tribulus* either cultivated or growing naturally at Onderstepoort has failed to cause geeldikkop in susceptible Merino sheep.

From experiments conducted with haematoporphyrin, various fluorescent dyestuffs, e.g. eosin, and also two species of the plant *Hypericum*, it was clearly shown that, although the resultant photosensitivity shown by the animals closely simulated that normally seen in true geeldikkop, the other important clinical symptom, viz., the intense generalised icterus normally seen in geeldikkop, was uniformly absent from the experimental cases. The blood serum in geeldikkop is usually of an intense clear yellow colour, giving a strongly positive direct Van den Bergh reaction. This suggests that the icterus is due to some form of obstruction possibly in the liver itself seeing that the bile duct leading to the duodenum is always found patent in affected cases.

In order to ascertain how this icterogenic factor operates, a series of experiments was started in which the bile ducts of sheep were ligated and the subsequent clinical symptoms carefully noted.

Experimental ligation of the bile duct in various animals has frequently been resorted to in connection with problems of digestion, icterus, functions of the gall bladder and the liver. In a recent paper Cameron and Oakley, besides reviewing the extensive literature on the subject, gave a detailed account of their work on rats. They showed that ligation of the common bile duct in the rat was followed by definite pathological changes in the liver. The animals were killed at hourly intervals and sections of the liver examined. Their results were compared with those obtained by other workers on such animals as the guinea-pig, rabbit, dog, cat, pigeons and mice, and from this it was shown that the type of liver response varied fairly widely in the different species of animals. From the available literature, it would seem that no systematic work has been done on the effect of ligation of the bile duct in sheep. For this reason the above-mentioned series of experiments were started on sheep. It was hoped that ligation of the bile duct would throw some light on the occurrence of icterus in natural cases of geeldikkop.

LIGATION OF THE BILE DUCT IN THE SHEEP.

Operative Procedure.

Under natural conditions it would appear that young Merino sheep are more susceptible to geeldikkop than full-grown ones. For this reason young sheep (under 18 months) were selected for operation as far as these were available. They were usually starved for 24 hours, during which time they were closely shorn along the whole length of the back and also the right flank. Immediately before the operation, a small sample of blood (25 c.c.) was drawn from a jugular vein and collected in a little sodium citrate. This blood was subsequently centrifuged and the percentage red cells ascertained, as also the colour of the plasma. Depending upon the size of the animal, 3 to 4 grams chloral hydrate in 10 per cent. saline solution was injected very slowly into the jugular vein. In this way complete general anaesthesia was usually obtained within 5 or 10 minutes. The right flank, previously shorn, was then shaved clean with a razor, using soap and water to soften the wool. Barium sulphide depilatories were found to be unsatisfactory as they invariably caused hyperaemia and irritation of the sensitive Merino skin. After this, the animal was placed on its left side on the operating table and the feet tied down. The skin over the site of the operation was then flushed down with ether. For the rest, full aseptic precautions were taken during the operation. A laparotomy wound usually 4 to 5 inches long was made parallel to and about 1 inch behind the last rib, the wound being kept open by retractors and artery forceps. By drawing aside the small intestines the visceral surface of the liver, the gall bladder and the extrahepatic bile ducts came into view. Overlying and very close to the vena portae, the ductus choledochus could be made out. This was carefully cleaned from the vena portae for a short distance immediately distal to the junction of the cystic duct. In the first few operations a single silk ligature was placed tightly round the ductus choledochus at this point. However, this procedure soon had to be changed, as it was found that, although clinical icterus made its appearance after a few days, it was of a definitely transitory nature and completely disappeared after 7-10 days. Such animals usually made an uneventful recovery with very little loss in their general condition. Several such animals were subsequently slaughtered for post-mortem examination, and in each case it was found that the bile duct had reconstructed itself round the ligature, which was found in the lumen of the enlarged portion of the duct. In this way the biliary circulation had become completely re-established. As mentioned by Cameron and Oakley, a similar observation was made by Brodie (1823) following ligation of the common bile duct in cats.

Thus, in order to obviate the bile duct from reconstructing itself, it was decided to place double silk ligatures about ¼ inch from each other and then to sever the duct between the two. In all subsequent operations this was done. On cutting the duct, it was found that the two stumps retracted, leaving a gap of about ½ inch. In this way the chance for union of the ends was minimised. This was borne out in practice as in no case did the duct become patent again. In addition to ligation and cutting of the ductus choledochus, the cystic duct too was ligated at its entry into the gall bladder and the bile removed from the gall bladder by means of a syringe and needle. This was done in order to prevent bile being forced in large amount into the gall bladder and so possibly causing rupture of its wall. By these means it was attempted to cause as little bile as possible from actually leaving the liver in the extrahepatic bile tracts. The laparotomy wound was closed by three layers of gut sutures applied to the peritoneum, muscles, and skin respectively, and dressed with iodoform and collodion.

All operated animals were placed in a clean stable for 24 hours, during which time only a little green feed was allowed. In practically every case, healing of the wound by first intention took place. From the second day onwards, the animals were regularly kept exposed to sunlight in a paddock daily from 9 a.m. to 4 p.m. In addition, a small volume of blood was collected in citrate every morning and the animals kept under frequent observation. The diet consisted of green lucerne, dry veld hay and a little crushed maize with water *ad lib.*

CLINICAL SYMPTOMS FOLLOWING LIGATION OF THE BILE DUCT. PHOTOSENSITISATION ESTABLISHED.

In these experiments a total of 51 animals were operated upon. Without exception the operation was withstood very well, although the period of survival afterwards varied widely. The blood plasma as taken just prior to the operation, was generally found to be water clear, or very faintly yellowish, although not sufficient to yield a positive Van den Bergh reaction.

A description of some of these cases will serve to indicate the type of response obtained.

Angora Goat No. 25303.

12.4.32—ductus choledochus ligated and cut. Cystic duct ligated. Serum clear.

13.4.32—animal lively, placed in sun, serum very yellow, no clinical icterus. Serum gives positive direct Van den Bergh reaction.

16.4.32—serum intensely yellow, clinical icterus as shown in mouth and conjunctivae.

24.4.32—animal dull, icterus intense.

25.4.32—marked photosensitisation after one hour in sunlight, scratching ground, marked flinching, ears slightly swollen, pits on pressure.

16.4.32—ears badly swollen and pendulous, eyelids swollen, eyes partly closed (see photo 1), marked flinching (see photo 2), very restless, urine deep yellow brown. (Weather: sky partly overcast, sun bright and hot.)

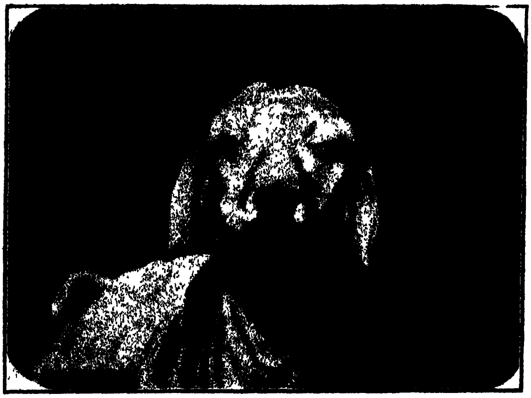

Fig. 1. Goat 25303. Head and ears swollen following ligation of bile duct.

27.4.32—condition unchanged, animal feeding fairly well.

28.4.32—swelling of ears subsiding, slightly sensitive in sunlight, serum still very yellow.

30.4.32—not sensitive, all swellings subsided, serum very yellow, still giving very strong direct Van den Bergh reaction.

3.5.32—animal died during night.

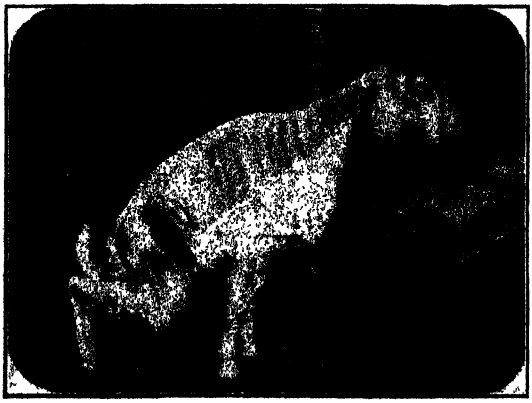

Fig. 2. Goat 25303. Acute photosensitisation following ligation of bile duct.

Post-mortem Report.—Emaciation, anaemia, severe generalised icterus, laparotomy wound healed. Bile ducts markedly distended with inspissated bile. Swelling and bile pigmentation of the liver and kidneys. Stasis in fore-stomachs and distal portion of large intestine.

Merino Sheep No. 33094.

24.6.32—ductus choledochus ligated and cut. Cystic duct ligated. Serum yellowish before operation.

25.6.32—animal lively, serum deep yellow, strong direct Van den Bergh test. Conjuctivae yellow.

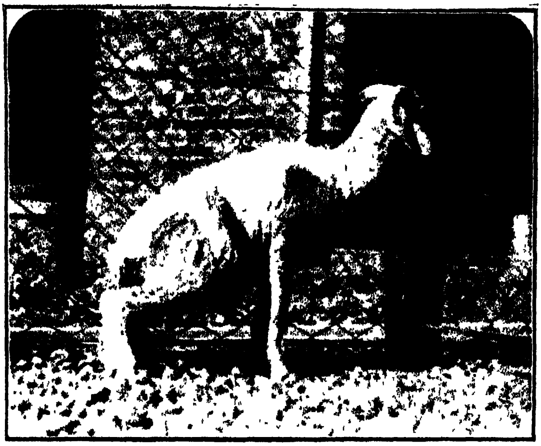

Fig. 3. Sheep 33091. Marked flinching after ligation of bile duct.

27.6.32—well marked photosensitisation, serum very yellow.

28.6.32—marked clinical icterus, urine yellow. Animal very sensitive in sunlight, crouching, biting its back and frequently sitting down on haunches, scratching the ground. Ears swollen (see photos 3, 4 and 5).

4.7.32—swelling subsiding, otherwise no change.

7.7.32—still sensitive to sunlight.

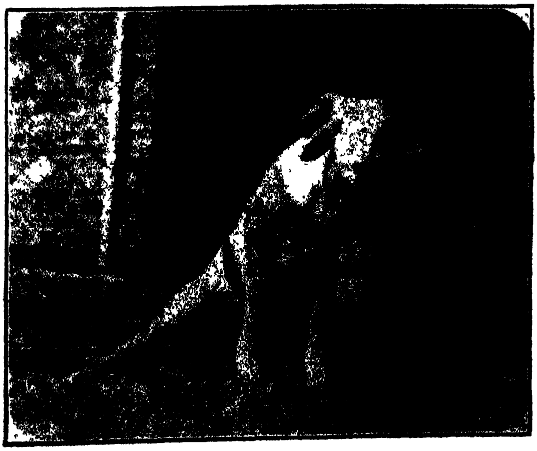

Fig. 4. Sheep 33094. Marked flinching after ligation of bile duct.

14.7.32—animal losing condition, although feeding well, marked irritation of the head to sunlight. Serum very yellow. Strong direct Van den Bergh test.

21.7.32—condition poor, still sensitive.

23.7.32—feet very painful, animal lame, coronets red.

30.7.32—markedly sensitive, animal crawling on knees. Eyelids and lips hard.

2.8.32—animal in extremis, lying on side, unable to rise. Died at 11 a.m.

Post-mortem Report.—Intense generalised icterus, anaemia and general atrophy. Oedema of the subcutis of the head and necrosis of skin. Laparotomy wound healed. Severe bile stasis in liver with marked widening of bile canals which on the surface of the liver appear like enlarged gall bladders. Bile pigmentation of the kidneys. Slightly pasty pellet-shaped faeces in the large intestine.

Note.—Throughout the course of the obstruction of the bile ducts the icterus index varied between 30 mg. and 50 mg. bilirubin per litre plasma as determined by the direct van den Bergh reaction.

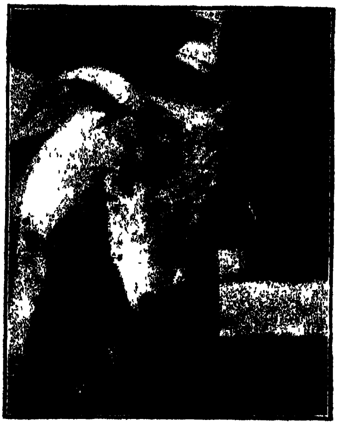

Fig. 5. Sheep 33094. Head and ears swollen after ligation of bile duct.

Merino Sheep No. 32577.

18.10.32—ductus choledochus ligated and cut, cystic duct ligated.

19.10.32—slight flinching noticeable at 4.30 p.m., also slight clinical icterus.

20.10.32—flinching marked when animal placed in sun.

22.10.32—animal very sensitive and flinching. Clinical icterus (conjunctivae) definite.

24.10.32—animal not sensitive, icterus marked.

31.10.32—condition very poor, animal unable to rise.

1.11.32—animal in extremis. Killed at 3 p.m.

Post-mortem Report.—Cachexia, anaemia, marked generalised icterus, dilatation of bile ducts, atrophy of gall bladder, pigmentation of liver and kidneys, stasis in fore-stomachs.

Merino Sheep No. 32542.

18.10.32—ductus choledochus ligated and cut. Cystic duct ligated.

19.10.32—animal pawing ground, slight flinching.

20.10.32—marked flinching, restless, slight clinical icterus, serum intensely yellow. Strong direct Van den Bergh test.

22.10.32—definite clinical icterus.

24.10.32—very sensitive, restless.

31.10.32—marked photosensitisation and flinching as soon as placed out in sunlight. Animal collapsed after 1 hour in sunlight. Placed in stable. In extremis. Killed at 3 p.m.

Post-mortem Report.—Cachexia and anaemia, intense generalised icterus, oedema of subcutis of head and ears. Marked bile stasis in liver. Superficial bile duct filled with colourless slightly turbid liquid. Pigmentation of the kidneys. Catarrhal enteritis and stasis in the large intestines.

Merino Sheep No. 32980.

30.6.32—ductus choledochus ligated and cut. Cystic duct ligated.

9 a.m.—before operation, serum water clear.

10.40 a.m.—immediately after operation, serum water clear.

12 p.m.—serum pale yellowish, faintly positive direct Van den Bergh test. Approximately 0·9 mg. per litre plasma.

1 p.m.—serum more yellow, 2 mg. bilirubin per litre.

2 p.m.—serum more yellow, 2+ mg. bilirubin per litre.

4 p.m.—serum quite yellow, 3 mg. bilirubin per litre.

1.7.32—24 hours after operation serum very yellow, 18 mg. bilirubin per litre plasma.

2.7.32—48 hours after operation serum deep yellow, 31 mg. per litre plasma, clinical icterus just starting.

5.7.32—no photosensitisation yet. Clinical icterus well marked, 55 mg. bilirubin per litre plasma.

9.7.32—animal very constipated, still no photosensitisation, icterus well marked.

11.7.32—animal died during the night.

Post-mortem Report.—Severe generalised icterus, bile stasis in liver with enlarged bile ducts. Intussusception large colon.

DISCUSSION.

From the description of a few of the large number of cases in which complete surgical obstruction of the bile flow had been carried out, it is evident that a well marked train of symptoms can be followed in the Merino sheep. Of these the first to be noticed is the very sudden rise of bile pigment in the circulating blood plasma. Thus in sheep 32980, where blood samples were taken at hourly intervals, a faintly positive direct Van den Bergh reaction was given within 80 minutes after operation. This is continued by a progressive rise in the bilirubin content of the plasma, although actual clinical icterus as noticeable on the skin, mouth and other mucous membranes, only follows after the lapse of some days. In the meantime a large amount of bile pigment is voided in the urine which may be coloured a deep yellow, and also giving a positive direct Van den Bergh reaction. This indicates an effective participation of the kidneys in eliminating some of the increasing amounts of bile pigment. The elimination via the urine is, however, incomplete, seeing that gradually an intense bile staining of all tissues follows, resulting in the well-marked clinical jaundice. Judging from the degree of bile pigmentation of the kidneys (macroscopically and microscopically) it would appear that after some days the kidneys become charged with an excessive amount of bile, all of which cannot be eliminated in the urine. The prolonged bathing of the body tissues in fluids rich in bile pigments then gives rise to the intense jaundice. It is obvious, however, that the liver retains its efficiency for secreting the bile into the normal bile channels, as shown by the tremendous distension of both the intra- and extra-hepatic bile ducts even up to the point of rupture, and also by the comparative ease with which the ligated ductus choledochus is re-established when one ligature only is applied around it. It would appear that no effort is spared on the part of the liver in forcing the increasing amounts of bile into the normal channels. In the liver itself a proliferation of the bile capillaries is frequently noted.

Accompanying the icterus, another symptom which is of unusual interest, is also developed, viz., that of photosensitisation. As far as can be ascertained, this symptom following bile duct obstruction, has not been described hitherto. The onset of photosensitisation varies widely. Thus it may appear on the second or third day after the operation, or it may be delayed for 12 or 14 days, while in rare cases as e.g. in sheep 32980 it may not be observed at all. The majority of animals, however, when closely shorn along the back and head, and kept exposed in sunlight, develop well marked symptoms of photosensitisation within the first week after the operation. That the condition is definitely associated with sunlight is shown by the fact that affected animals continually seek for shade. Furthermore, when the sheep are kept in a stable, no sign of photosensitisation is shown, whereas if they are placed out in the sun, licking of the lips, scratching of the head and ears, marked flinching and restlessness may be observed within a few minutes to a few hours. At times the irritation is so intense that the most abnormal attitudes are adopted, e.g. sitting up on the haunches or dragging the hind legs along, with the abdomen held close to the ground (see photographs). Following on this, oedema of the exposed parts, e.g. ears, face, lips and peri-orbital region rapidly develops, and so causes a peculiar round appearance of the head, with the ears thick and pendulous, and the eyes closed. The height of the oedema is usually reached within 24 hours. Subsequently it gravitates towards the intermandibular space, which becomes bag-like in appearance. With the subsidence of the oedema a change in the affected skin can be noticed. At first it is tough and leathery and of a dull greyish yellow colour, especially so along the shorn back. The base of the horns and the feet round the coronet frequently appear red and painful. The animal may be very sensitive on the feet, or quite lame and disinclined to stand. In no case were those parts of the skin which were fully protected by a covering of wool found to be affected; the demarcation between diseased and healthy skin corresponding exactly with the shorn and unshorn parts. After some days the affected skin becomes darker and frequently of a greenish tint. At the same time it hardens to the consistence of dried leather and easily cracks. Unless death supervenes too quickly, extensive sloughing of the necrosed layers of the skin and its wool may take place, so exposing the newly formed healthy pink skin. In rare cases the tips of the ears may slough completely.

The actual cause of this photosensitisation has not been ascertained as yet, although it is reasonable to suggest that it is brought about by some fluorescent substance, seeing that it is generally assumed that this phenomenon is only caused by fluorescent bodies. Furthermore, it would appear that following the obstruction of the bile flow, this photosensitising substance which may even be a normal constituent of bile, increases in the circulation as the jaundice becomes more marked. In this connection some functional disturbance of the liver may be of primary importance. In the presence of sunlight and with an unpigmented and exposed skin, the photosensitising principle is rendered capable of producing the symptoms and lesions typical for photosensitisation as, for example, seen with haematoporphyrin.

Another symptom which needs explanation is the marked loss of condition of the animal, in spite of the fact that the appetite remains good after the operation. Furthermore, there are no obvious signs of digestive disturbance except towards the end, when constipation in the caecum and upper portion of the large intestine is frequently noticed. Prior to this, the faeces appear normal, although the bile is prevented from playing its usual part in digestion. With a normally low intake of fats in the food, it would appear that in the sheep the presence of bile in the intestine is of less importance than e.g. in the dog, although such factors as absorption from the intestine, metabolism in the liver and detoxification, may also be profoundly influenced in sheep when the bile flow is obstructed. These factors may actually be responsible for the loss of condition.

From the above description and discussion of the results obtained after simple obstruction of the bile flow in Merino sheep, it is evident that the symptom complex in many respects resembles that seen in true geeldikkop, although in the latter condition the skin lesions are generally far more severe, e.g. as shown by complete loss of ears, total blindness, extensive and deep necrosis of the facial skin causing immobility of the lips, jaws and eyelids. Thus, in an attempt to accentuate the symptoms following simple bile duct obstruction, various other measures were subsequently introduced either simultaneously or after the operation. The following account discloses the results obtained in this series of experiments, the term " bile ducts obstructed " being used to indicate that the above-mentioned operation had been performed, i.e. ductus choledochus ligated and cut and the cystic duct ligated at the neck of the gall bladder:—

1. *Bile ducts obstructed and gall bladder removed.*—This operation was performed on 5 sheep. Four of these sheep died within 7 days after operation without showing photosensitisation, although the icterus was well marked. The remaining one sheep showed typical photosensitisation two days after operation. On the 4th day the ears

were markedly swollen and icterus visible. It died 13 days after operation. Of the 5 sheep, 4 showed a well marked bile peritonitis on post-mortem due to rupture of the superficial bile ducts of the liver and bile seepage from the liver surface where the gall bladder had been removed.

It may thus be concluded that the additional removal of the gall bladder offers no advantages over the ordinary operation, while death from bile peritonitis frequently ensues in such cases.

2. *Partial obstruction of the Vena portae.*—The object in this case was to ascertain whether a decreased supply of portal blood to the liver could possibly lead to functional derangement of the liver resulting in icterus and photosensitisation.

One Merino sheep was operated on through the right flank, and a silk ligature placed loosely round the vena portae about one inch below its entry into the liver. The ligature was adjusted so as to reduce the lumen of the portal vein to about $\frac{1}{3}$ to $\frac{1}{4}$ of its normal. The animal made an uninterrupted recovery after the operation. It was placed out in the sun and kept under close observation for 12 days. No symptoms whatever were noted and the serum also remained water clear. The general health and feeding remained good throughout. On the 13th day another laparotomy operation was performed immediately posterior and parallel to the first one. On opening the peritoneal cavity, large masses of calcified omental fat were found. In addition, a large network of newly-formed veins were made out. These veins connected the omentum with the parietal peritoneum and ran in a forward direction. As it was impossible to reach the ligature through these veins, the wound was again closed. Complete recovery again ensued, while the general health remained good. It would appear that due to the partial obstruction in the portal vein, compensation rapidly set in by the formation of new veins from the omentum to the abdominal wall, so ensuring a proper return of portal blood into the systemic circulation.

3. *Partial obstruction of the posterior Vena cava.*—Since obstruction in the portal vein did not yield the required symptoms, it was decided to ascertain the effect of partial occlusion of the posterior vena cava immediately above the liver. This was firstly attempted through an abdominal wound, although this gave great difficulty on account of the close adherence of the liver on to the diaphragm in the region of the great veins and also because of the posterior vena cava actually penetrating through the dorsal part of the liver. Consequently on another sheep, the operation was attempted through the thoracic cavity with the animal under artificial respiration. A silk ligature was tied loosely round the posterior vena cava immediately anterior to the diaphragm and its lumen reduced to about $\frac{1}{3}$ the normal. The operation was withstood well and the animal quickly recovered. The only symptom noticeable for several weeks after the operation was respiratory distress, the sheep panting vigorously, especially when chased around the paddock. As no further symptoms developed, the animal was killed for post-mortem examination 37 days after the operation. The most important findings included a marked calcification of fat all over the body, severe ascites amounting to 6·5 litres, cardiac dilatation, atrophy and well-marked cirrhosis of the

liver. The digestive tract appeared normal. Thus the result of this experiment was simply what was to be expected in a case of chronic venous stasis in the liver and without any secondary symptoms of icterus or photosensitisation.

4. *Bile ducts obstructed and hepatic artery ligated.*—It was hoped that in addition to the usual results obtained with obstruction of the bile flow, interference with the arterial blood supply to the liver might lead to more severe symptoms of icterus and photosensitisation. Consequently in two sheep the hepatic artery was included in the ligatures round the ductus choledochus and completely obstructed. In one sheep photosensitisation was noticed 5 days after the operation, while the other one never showed this symptom. In both cases, however, icterus was intense. Both animals died, the one after 6 days and the other after 21 days with lesions similar to those found in ordinary bile obstruction cases.

It may thus be stated that the symptoms remain the same whether the hepatic artery is ligated or not.

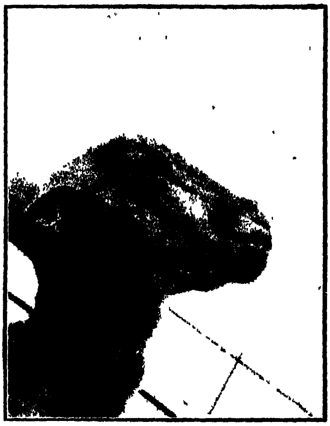

Fig. 6. Sheep 32645. Head and ears swollen after ligation of bile duct, hepatic artery, splenic artery and vein.

5. *Bile ducts obstructed and spleen removed.*—In order to ascertain what rôle the spleen plays in the production of obstructive jaundice and photosensitisation, various operations were carried out.

In one sheep the spleen was removed at the same time that the bile ducts were obstructed. No photosensitisation was shown by this animal, although the icterus was very marked. Death occurred on the 18th day with marked bile stasis in the liver.

In one sheep the splenic vein was ligated and the bile ducts obstructed. Photosensitisation was well marked within 24 hours after operation. On the third day the animal was flinching badly and the ears were markedly swollen. By the 8th day icterus was marked and the animal still sensitive in sunlight. The serum showed a very strong direct van den Bergh reaction, amounting to 125 mgm. bilirubin per litre. Death occurred on the 18th day. The lesions found included a marked generalised icterus, hard crusts round the lips and nostrils, bile ducts greatly enlarged, swelling and pigmentation of the liver, enlargement and degeneration of the spleen.

In another sheep the bile ducts were obstructed and at the same time the hepatic artery was ligated and also the splenic vein and splenic artery ligated. No symptoms were shown up to the 9th day, when clinical icterus was visible. On the 15th day the animal suddenly became very sensitive to sunlight and the ears and lips very much swollen (see photo 6). On the 16th day the eyes were practically closed and the oedematous conjunctivae protruding. The animal remained very sensitive up to the 21st day, when it died, the main lesions being a generalised icterus, swelling and pigmentation of the liver, marked enlargement of the bile ducts and formation of a diverticulum, swelling of spleen, necrosis of lips and round about eyes. All ligatures were found to be intact.

From these cases it is evident that the symptoms of icterus and photosensitisation may develop without the normal function of the spleen.

6. *Bile ducts obstructed and one kidney removed.*—The object of this experiment was to note whether in the absence of one kidney, the icterus and photosensitisation would be increased, seeing that large amounts of bile pigment are voided in the urine in obstruction icterus cases. In two sheep the right kidney was removed, after ligation of the renal vessels, at the same time that the bile ducts were obstructed. The operation was stood well, the animals soon recovering from the effects. One sheep became very sensitive to sunlight on the third day. The following day the ears were markedly swollen and a slight icterus was noticeable. Thereafter the animal gradually lost its condition while the photosensitisation became less marked. It was killed in extremis 4 weeks after the operation. The carcase was emaciated, bile stasis in the liver, left kidney slightly enlarged and bile pigmented. The other sheep became sensitive on the 4th day after operation. On the 5th day it was flinching badly, the feet were tender and the animal disinclined to move. The ears, face and lips were swollen and a slight icterus was present. The animal remained sensitive up to the 16th day, when it died, the main lesions being marked icterus, slight cirrhosis and bile pigmentation of the liver, slight hypertrophy of the remaining kidney, obstruction in the caecum.

From these two cases it would appear as if the removal of one kidney does not intensify the symptoms obtained with simple biliary obstruction.

7. *Bile ducts obstructed and intestine partially occluded.*—In this series of experiments the object was to ascertain whether obstruction in the intestines at different levels would accentuate the symptoms produced by simple bile duct ligation. The operation was carried out by placing a small aluminium band round the intestine and then by means of pliers bending in the ends to form a ring round the intestine. In this way various degrees of constriction could be produced. In one sheep the ring was placed round the duodenum six inches behind the pyloric sphincter and the lumen reduced to about ⅓ the normal. Photosensitisation was first observed 5 days after the operation. The following day the animal showed marked flinching and restlessness with a rapidly developing oedema of face, ears, and

lips (see photos 7 and 8). The next morning the swelling had gravitated and a large bag-like swelling was noticed between the lower jaws (see photo 9). On the 8th day the swelling started to subside. There was no sign of clinical icterus as yet, although the serum was clear yellow. The animal was feeding badly and losing condition. Faeces collected appeared normal. On the 10th day the sheep still showed marked flinching as soon as it was placed out in the sun. On the 13th day it collapsed and was killed in extremis. The post-mortem revealed cachexia, marked necrosis of the skin over the face, ears and lips, swelling of the liver with marked distension of the bile ducts. The ring on the duodenum, although practically occluding the lumen did not provoke a stasis probably because of the fluid consistence of the chyme. In another sheep a ring was placed on the ileum 6 inches above the caecum. The animal died 4 days later after showing symptoms of abdominal pain and great thirst. On post-mortem a large amount of water was found in the rumen and small intestines. Due to rupture of the bile duct above the ligature bile peritonitis had supervened.

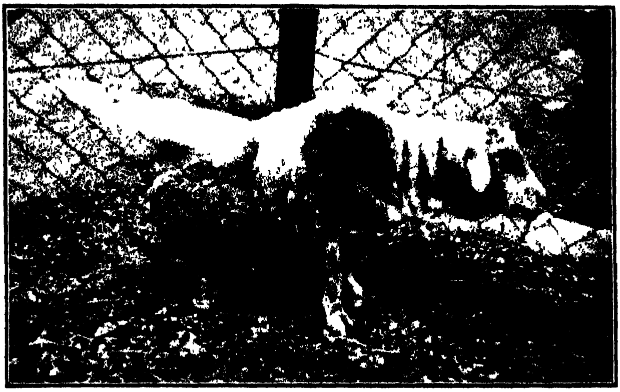

Fig. 7. Sheep 35353. Marked flinching after ligation of bile duct and closure of duodenum.

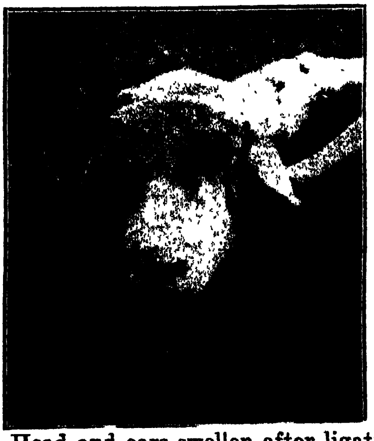

Fig. 8. Sheep 35353. Head and ears swollen after ligation of bile duct and closure of duodenum.

Obstruction of the large intestine was produced in two sheep by placing a ring just posterior to the ansa spiralis. Both animals became dull after the operation and remained in this state. No photosensitisation was shown by either of them. One sheep died on the 7th day with intense generalised icterus, haemoglobinuria, sub-epicardial haemorrhages, swelling and necrosis of the liver, bile ducts distended, enlargement of the spleen and kidneys, stasis in the colon above the ring. The lesions suggested an acute toxaemia following complete obstruction in the large intestines.

The other sheep died on the 20th day, showing similar lesions to the one above, although less intense.

8. *Bile duct obstruction followed by ultra-violet light irradiation.*—In order to ascertain whether ultra-violet rays were responsible for the photosensitisation, two sheep after being operated, were exposed at a distance of two feet to a quartz mercury vapour lamp 30 minutes daily for 5 days. This procedure had no effect whatever on the animals. On the 6th day the animals were exposed to sunlight, with the result that both showed well marked photosensitisation within 30 minutes. Both animals died on the 14th day after operation, remaining sensitive to sunlight up to the time of death. In both, lesions of bile stasis, enlargement of the liver and icterus were noticed.

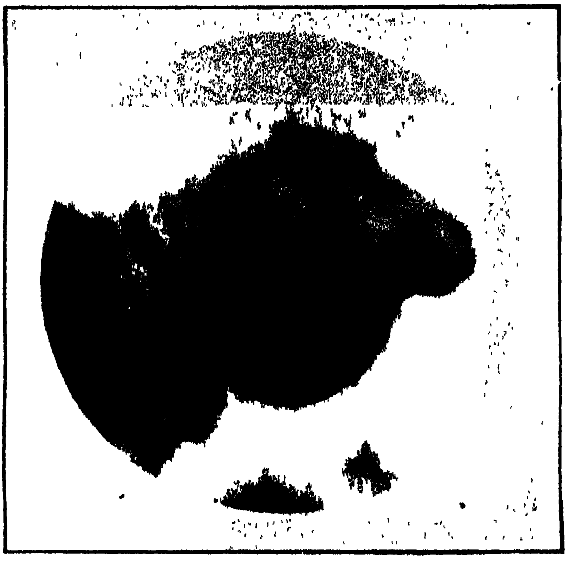

Fig. 9. Sheep 35353. Swelling gravitated after ligation of bile duct and closure of duodenum.

9. *Bile duct obstruction combined with inhalation of arsenuretted hydrogen.*—By allowing operated animals to inhale arsenuretted hydrogen, it was hoped that the well marked haemolysis produced by this poisonous gas might provide the bile forming tissues with extra

amounts of free haemoglobin and so possibly increasing the severity of the jaundice, and the photosensitisation. Two operated sheep were forced to inhale arsenuretted hydrogen for ½ minute on two consecutive days, starting on the 2nd day after the operation. The sheep were bled immediately after each inhalation and the serum examined spectroscopically. In each case the bands ot oxyhaemoglobin were seen. Thereafter the animals were exposed to sunlight. One sheep showed marked flinching and swelling of the head on the 4th day after the operation. This continued up to the 7th day. On the 8th day the swelling had gravitated to the region between the lower jaws, which appeared bag-like and filled with fluid. The eyes remained partly closed. Icterus was intense, even the tears and the saliva assuming a deep yellow colour (see photo 10). The animal was killed the same day. The post-mortem revealed a slight anaemia, generalised icterus, bile pigmentation and swelling of the liver and kidneys, swelling of the spleen. The other sheep showed haemoglobinuria within a few hours after inhalation of the arsenuretted hydrogen. The conjunctivae assumed a dark brown colour. Six days after the operation the sheep became sensitive to sunlight, showing marked flinching and swelling of the ears. The conjunctivae were intensely yellow and the serum deep brownish yellow. By the 12th day the swellings had subsided, while the skin over the face and ears was hard and dry. On the 14th day marked photosensitisation was again shown, accompanied by some swelling of the ears. The animal was in poor condition and feeding badly as a result of the hardness of the lips (see photos 11, 12 and 13). It was killed on the 20th day after operation. On post-mortem there were lesions of cachexia, anaemia, well marked necrosis of lips, ears and facial skin, enlargement and bile pigmentation of the liver and kidneys, stasis in the fore-stomachs and large intestines.

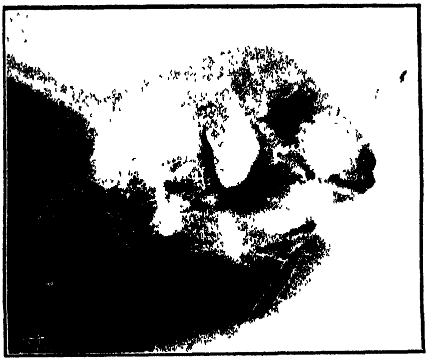

Fig. 10. Sheep 35353. Head and inter-mandibular region swollen after ligation of bile duct and inhalation of arsenuretted hydrogen.

From the above cases it would appear as if the inhalation of arsenuretted hydrogen did provoke more intense symptoms of icterus and photosensitisation.

10. *Bile ducts obstructed under pure choloform anaesthesia followed by repeated administration of chloroform.*—Seeing that chloroform may act as a powerful liver poison, it was thought that its use as an anaesthetic may cause more pronounced symptoms in cases of bile duct obstruction. Consequently, several sheep were operated under pure chloroform anaesthesia. Thereafter the animals received daily injections subcutaneously of 0·5 c.c. chloroform in liquid paraffin. In one sheep photosensitisation was seen on the 3rd day after operation. On the 6th day there was marked flinching and clinical icterus. This continued up to the 16th day, when the ears were noticed to be hard and dry. The animal died on the 20th day with lesions of intense icterus, necrosis of the ears, swelling, pigmentation and degeneration of the liver and kidneys, stasis in the fore-stomachs. The other three animals died within the first week without showing photosensitisation. Thus it appears that chloroform does not greatly intensify the symptoms produced by simple biliary obstruction.

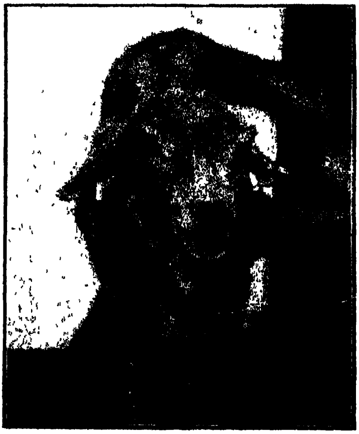

Figure 11.

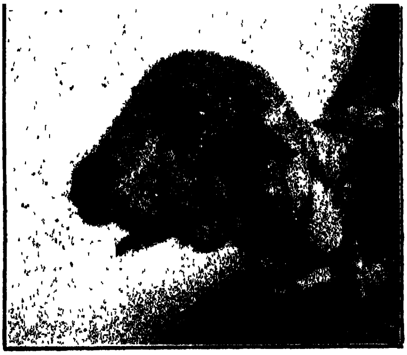

Figure 12.

Fig. 11. Sheep 35351. Necrosis round eyes and lips after ligation of bile duct inhalation of arsenuretted hydrogen.

Fig. 12. Sheep 35351. Sloughing of skin round eyes and nostrils after ligation of bile duct and inhalation of arsenuretted hydrogen.

11. *Bile ducts obstructed, followed by oral administration of carbon tetrachloride.*—Due to the toxic action of carbon tetrachloride on the liver it was decided to attempt to intensify the liver disturbance by repeatedly dosing it to operated sheep. One sheep was dosed for 4 weeks with a total volume of 228 c.c. carbon tetrachloride dissolved in olive oil, starting with 4 c.c. carbon tetrachloride daily and ending with 20 c.c. daily. The animal became slightly sensitive to sunlight on the 6th day after operation, by which time clinical icterus was also visible. The serum was deep yellow and gave a strongly positive direct Van den Bergh reaction. On the 15th day slight sensitisation was again noticeable. This, however, soon disappeared. Thereafter the animal became dull, and condition was lost until it was killed in extremis on the 31st day.

On post-mortem, the carcase revealed cachexia, anaemia, intense generalised icterus, necrosis of skin round the mouth, marked swelling, degeneration and pigmentation of the liver and kidneys with enlargement of the bile ducts, stasis in the fore-stomachs.

In this case carbon tetrachloride therefore produced no visible effect on the course of the condition, in spite of the large amount administered.

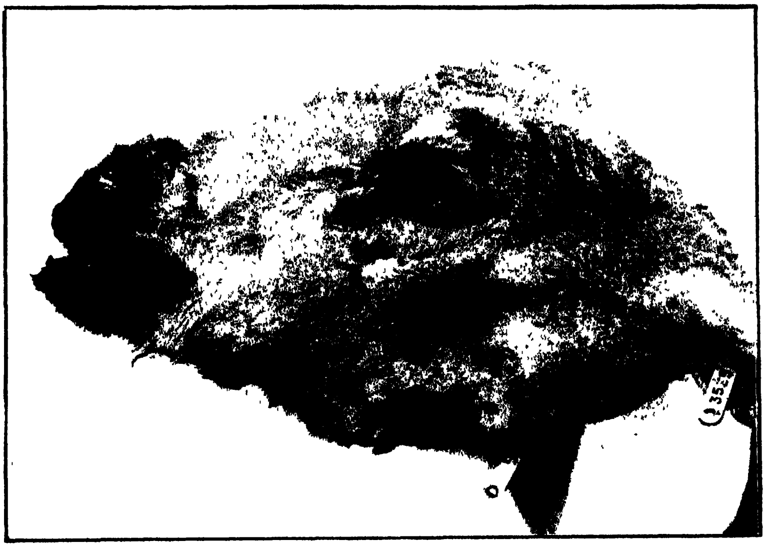

Fig. 13. Sheep 35351. Marked necrosis round eyes and nostrils after ligation of bile duct and inhalation of arsenuretted hydrogen.

12. *Bile ducts obstructed, followed by administration of toluylenediamine and chloroform.*—Since toluylenediamine is known to provoke well marked icterus in the dog, it was decided to test it out on an operated sheep in combination with chloroform. The animal

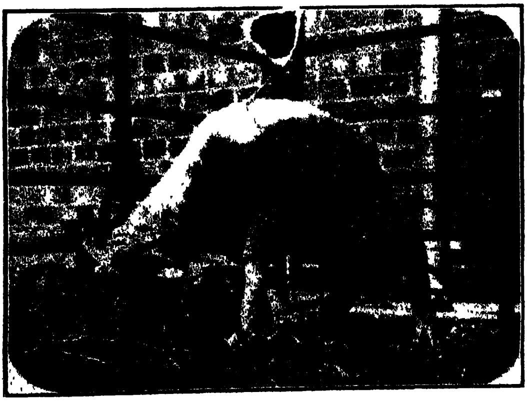

Fig. 14. Sheep 32638. Marked photosensitisation after ligation of bile duct and dosing with chloroform and toluylenediamine.

received daily doses of 0·5 gm. toluylenediamine and 0·5 c.c. chloroform (in water) by stomach tube. This was continued for a period of 12 days. The animal showed slight icterus on the second day, the urine being deep yellow. On the third day it showed flinching and the ears began to swell up. On the 4th day the sensitisation was very marked, the animal throwing its body into unusual attitudes (see photographs 14 and 15). On the 13th day the skin of the face and over the shorn back was noticed to be hard and cracking. The animal remained sensitive up to the 21st day, while the facial skin changed to a dirty greyish black and covered with hard crusts (see photo 16). The sheep had great difficulty in feeding and drinking, and was killed in extremis on the 23rd day after operation.

Fig. 15. Sheep 32638. Marked flinching (same as Fig. 14).

The post-mortem revealed cachexia, anaemia, necrosis of skin over the head and back, severe icterus, swelling, pigmentation and bile stasis of the liver with diverticula of the superficial bile ducts. swelling and pigmentation of the kidneys, slight swelling of the spleen, stasis in the fore-stomachs, and absence of food in the small intestine.

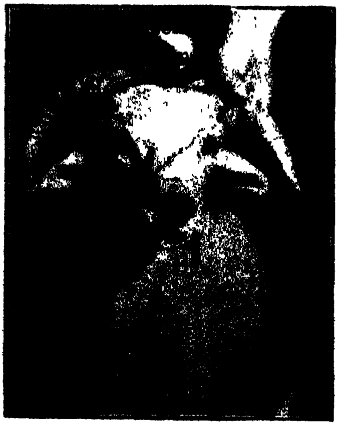

Fig. 16. Sheep 32638. Necrosis round eyes and nostrils (same sheep as in Fig. 15).

13. *Bile ducts obstructed, followed by administration of phenylhydrazin.*—Phenylhydrazin through its haemolytic action is known to produce icterus in the dog. It was therefore hoped that in the sheep it may increase the symptoms following obstruction of the bile ducts.

Into one operated sheep phenylhydrazin hydrochloride was injected intravenously (in saline solution) in daily doses of 0·1 gm. over a period of 12 days. On the 3rd day the serum was definitely yellow, while haemoglobin bands were faintly visible spectroscopically. On the 5th day the serum assumed a very brown colour, presumably due to the mixture of bile pigments and haemoglobin. The urine, too, was dark brown. The animal became slightly dull and somewhat weak in the legs, but no photosensitisation was shown. On the 9th day clinical icterus was well marked. From the 12th day onwards the serum changed to a pale yellow colour, while photosensitisation was still absent. The condition improved and after 4 weeks the animal was discharged with its serum only faintly yellowish.

14. *Bile ducts obstructed, followed by injections of manganese chloride.*—One operated sheep received daily intravenous injections of 0·1 gm. manganese chloride on three consecutive days. On the 4th day the animal became markedly sensitive to sunlight with flinching and subsequent swelling of the ears. On the following day the swelling of the ears and head had increased (seé photo 17), and the animal was intensely irritated. From the 7th day onwards the swellings decreased with clinical icterus definitely visible. By the 14th day the animal was very weak and death followed the same day.

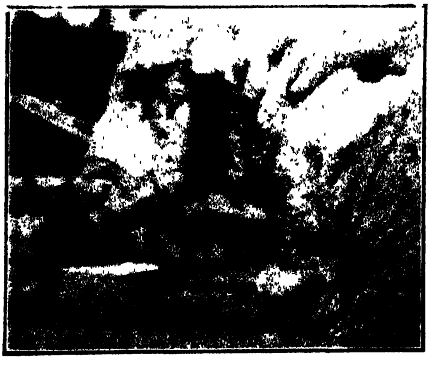

Fig. 17. Sheep 35334. Head and ears swollen after ligation of bile duct and administration of Manganese Chloride.

On post-mortem the carcase showed cachexia, necrosis of the skin over the face and lips, very marked icterus, swelling of the liver with dilatation of the bile ducts, marked swelling of the spleen and kidneys, stasis in the fore-stomachs.

15. *Bile ducts obstructed, followed by injections of* B. coli *cultures.*—In order to ascertain whether certain types of bacteria normally present in the digestive system, e.g. *B. coli* could, under certain conditions, play a rôle in the production of icterus and photosensitisation, several sheep were injected with 24-hour broth cultures of *B. coli.*

One sheep received 5 c.c. broth culture into the liver via the common bile duct immediately after it was ligated. The animal showed no symptoms until the 3rd day, when it became markedly sensitive to sunlight, flinching and very restless. On the 5th day the ears, nose and lips were markedly swollen (see photo 18). On the 7th day the animal was injected intravenously with 10 c.c. 24-hour broth culture of *B. coli*. By this time the animal was showing well marked icterus, while the feet appeared tender and painful. Death ensued that same night.

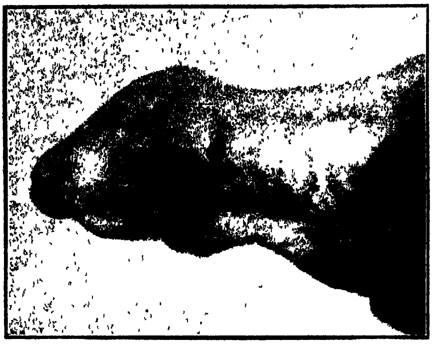

Fig. 18. Sheep 35323. Head and ears swollen after ligation of bile duct and injection of *B. coli* culture.

On post-mortem there was a well marked oedema of the subcutis of the head and ears, generalised icterus, pulmonary oedema, bile pigmentation of the liver and kidneys.

Another sheep was injected intravenously 5 c.c. broth culture of *B. coli* immediately after the operation. The following day the animal was dull, but the ears were distinctly swollen. On the third day the animal was markedly sensitive, and the ears very much swollen. There was no sign of clinical icterus as yet. On the 7th day the ears and face were still swollen, although the tips of the ears and lips were beginning to harden (see photo 19). There was a soft bag-

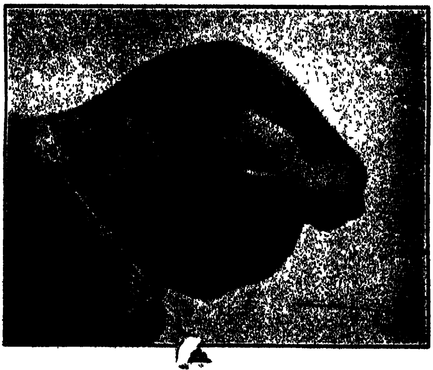

Fig. 19. Sheep 35338. Head and ears swollen after ligation of bile duct and injection of *B. coli* culture.

like swelling in the intermandibular space and clinical icterus distinct. Another intravenous injection of 10 c.c. broth culture of *B. coli* was again given. The animal died the following day, the main lesion on post-mortem being a generalised icterus, gelatinous infiltration of the subcutis of the head, ears and intermandibular space, pigmentation of the liver and kidneys and swelling of the spleen.

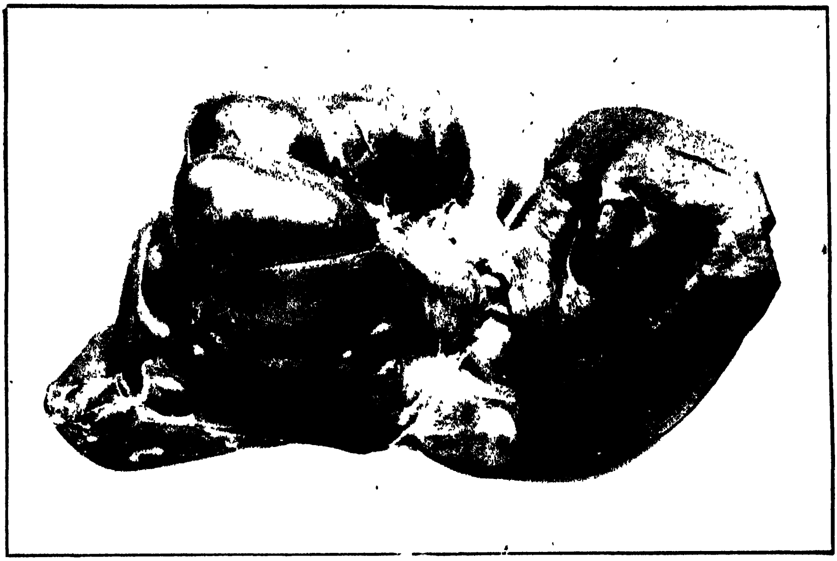

Fig. 20. Liver showing marked dilatation of superficial bile tracts following ligation of common bile duct and cystic duct.

SUMMARY.

1. The object in undertaking bile duct obstruction experiments in sheep, was to throw further light on the genesis of icterus and photosensitisation in true geeldikkop as caused by *Tribulus* spp. and also by other plants in South Africa.

2. The operative procedure was described for the ligation and obstruction of the extra-hepatic bile tracts in the sheep.

3. The ensuing clinical symptoms and the blood of operated animals were studied from day to day.

(*a*) A progressive bilirubinaemia was noted within one hour after operation. Clinical icterus, however, only presented itself several days afterwards and then persisted throughout the course of the condition. Within 24 hours the blood, and later also the urine, showed a strong direct van den Bergh reaction.

(*b*) Practically every animal, with the head and back closely shorn, when exposed to sunlight, developed symptoms of photosensitisation within one week of the operation. This was shown by the animals flinching, shaking the head and licking the lips. Soon afterwards oedema of the subcutis of the affected skin set in, e.g. the ears became thick and pendulous and the face swollen. Photosensitisation usually persisted for several days and in some cases lasted throughout the course of the condition. This was followed by hardening of the affected skin and sloughing of the superficial layers. Skin protected by a coat of wool remained normal.

(*c*) Animals with the biliary tract obstructed showed a progressive loss of condition up to the point of extreme emaciation.

4. On post-mortem the lesions found were those of intense generalised icterus, enlargement of the liver with marked bile stasis, dilatation of all the biliary tracts above the point of obstruction, enlargement and bile pigmentation of the kidneys, and frequently stasis in the large intestine.

5. Various modifications of the above operation were also attempted. The resultant symptoms and post-mortem lesions, however, were not altered or intensified to any extent.

Thanks are due to Mr. M. Carlisle for assistance rendered and to Mr. T. Meyer for taking the photographs.

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Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 1, Number 2, 1933.

Watery Whites of Eggs.
Report of Preliminary Investigations.

By A. S. CANHAM, F.R.C.V.S., Veterinary Research Officer, Allerton, Pietermaritzburg.

INTRODUCTION.

THE egg industry has in the last few years assumed great importance to South Africa. The result has been that more and more eggs have been exported overseas, and the poultry farmer has been able to augment his annual income somewhat.

Egg production can be divided among three groups of producers: (*a*) The man who keeps a few fowls in his back yard for egg production for his own domestic purposes; (*b*) the man who keeps a fair number of fowls for breeding high production strains of birds and for rearing day-old chicks for sale to (*c*) the poultry farmer proper who keeps large numbers of fowls intensively for the production of eggs for sale in the Union and for export overseas.

With high production come many problems. Eggs must be of such a quality that when the housewife buys them they must be fit to be eaten. They must be of such a quality that they will stand storing, being sent overseas, and still be fit for human consumption. In South Africa eggs for consumption are sold from the producer direct to the consumer or to a store or finally to the big co-operative egg circles for disposal. At the egg circles where very large numbers of eggs are handled all eggs are tested prior to sale to the public. Testing consists mainly in the use of the " candle ". By this means they have come to recognize in eggs such conditions as blood clots, meat spots, grass eggs, and " watery white " eggs, etc.

At the request of the Natal Co-operative Egg Circle we decided to investigate this last condition, especially as it appeared to be on the increase. Again, eggs that had passed the test here were found at the time of testing on arrival in England to show the typical " watery white " appearance.

DEFINITION OF CONDITION KNOWN AS " WATERY WHITE " EGG.

One has difficulty in drawing up a definition of this condition, and the description that is given below may have to be modified as our knowledge of the causation of " watery white " eggs increases.

However, as a definition of a term that is still used rather loosely, it is probably quite satisfactory. According to the wholesale egg trade firms it is defined as—

(1) an egg showing over the candle a trembling or vibrating membrane holding the albumin,

(2) an egg showing over the candle a rupture of this membrane with subsequent mixing of the albumin with the air of the air sac—bubbles may be seen or the entire air from the air space may move in one mass all round the egg, being always uppermost.

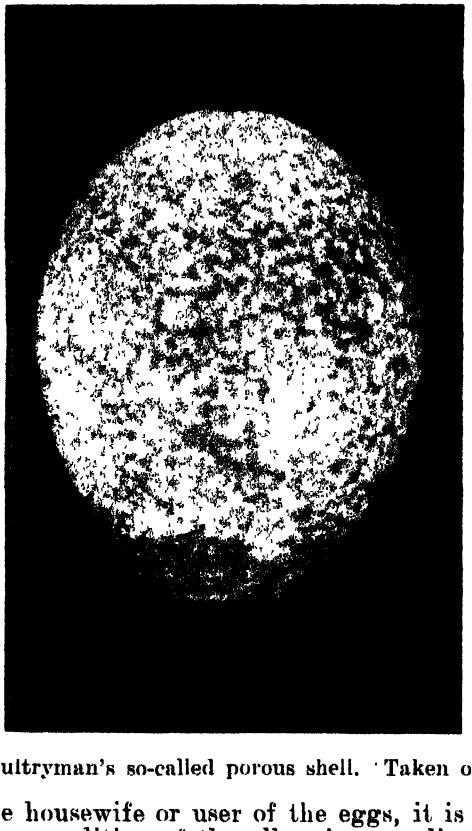


Fig. 1. The poultryman's so-called porous shell. Taken over the candle.

According to the housewife or user of the eggs, it is an egg showing (1) a very watery condition of the albumin, usually accompanied by a spreading yolk due to a weakened yolk membrane. These yolks frequently break when the eggs are poached; they in fact simulate a preserved egg. In some cases an egg showing a high percentage of dense albumin, which nevertheless flows more easily than usual, is also classed as a watery white egg.

To combine the views of both groups of people with our own one would state that a watery white egg is one which shows over the candle a tremulous or ruptured membrane holding the albumin, and which, when broken open, shows a watery appearance of the albumin associated with a weak yolk membrane.

There are, however, many eggs which, over the candle, show a normal appearance, but on being broken open may have the albumin and yolk sac affected, as described above; these should for the present, at any rate, also be regarded as watery white eggs. The wholesale egg trade firms rely mainly on the use of the " candle ". This statement is borne out by St. John, J. L., and Flor, I. H. (1931), who say: " As far as the wholesale trade is concerned ' watery whites ' seem to be identified almost entirely through candling ". In view of the results obtained in this work, it will be seen that candling is not an altogether reliable method for the detection of watery white eggs.

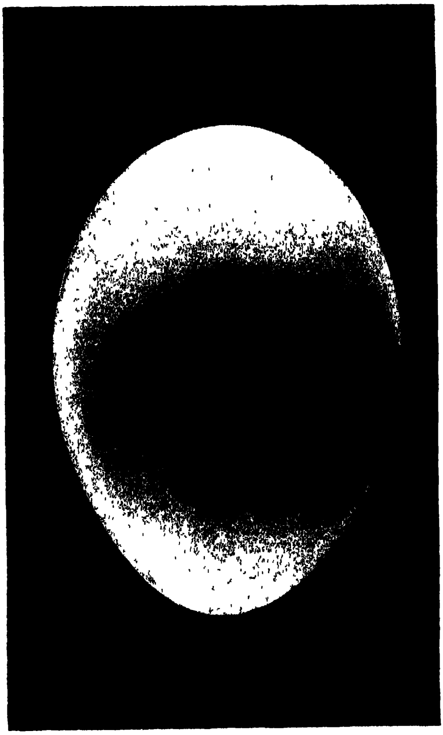


Fig. 2. Normal shelled egg. Taken over the candle.

GENERAL OBSERVATIONS.

This condition is very common in the Orange Free State and in Natal, in both places being noted by the writer; and it is quite likely that it is present throughout the Union. One will now and then see it commented on in the English poultry press.

One sees it at its height during the warm summer weather, although odd affected eggs occur the whole year through.

The egg circle in Durban, which is perhaps the chief depot of Natal, gets almost the major portion of its eggs from the outlying districts. These consignments usually have a trip by wagon first to the nearest siding or station; if they are before train-time, they are placed either under shelter or left in the sun. The eggs are packed in thick, wooden, felt-lined boxes, for the most part. Such cases are packed in hot vans, and have a short or a long journey to Durban. From Durban station they are carted by trolley, probably never covered against the sun's rays, to the egg circle. Many affected eggs reach this depot.

The egg circle at Pietermaritzburg caters more for the local egg producers, who, for the most part, live only a few miles away. These eggs are brought in two or three times a week in the producer's car and in it are sheltered from the direct rays of the sun. Only very few affected eggs reach this depot.

When one comes to consider the prevailing state of affairs on the poultry farm, a number of conditions are observed.

Many poultrymen have too few nests, with the result that if eggs are only collected once a day, a large number are kept hot for almost twelve hours by the different hens that enter the nests to lay their eggs. Where broody hens are not seen, a similar state of affairs prevails. Eggs, when collected, are not often cooled, but are placed in egg houses, which in summer are too warm and are not well ventilated.

The aim of the poultry-man these days is to obtain fowls that lay their 300 and more eggs per season, but they appear to lose sight of the fact that such a highly-producing " machine " hen must give in in time from the intense strain, despite adequate feeding.

LABORATORY INVESTIGATIONS.

(a) Description of Eggs which on Candling are known as Watery White Eggs.

Such an egg when examined prior to candling usually has a normal appearance. On moving it or shaking it slightly, one sometimes hears movement inside the shell; at times even a slight swish of liquid can be heard. On placing this egg on the " candle ", one sees no air space at the broader end, but a large bubble—or one large and several small bubbles—lying uppermost in the shell, according to how the egg is placed. Whatever way the egg is placed this bubble always remains uppermost.

Eggs of this description were X-rayed in order to make certain that the bubbles were air bubbles. This was checked by taking test tubes and filling two with the normal thin albumin part of an egg and two with the thick albumin part of an egg. All these tubes had at least one air bubble. These were X-rayed and then shaken well, so as to break up the bubbles. These were again X-rayed. Then watery white eggs showing one large bubble were shaken and X-rayed; and we found, similarly to the albumin in the test tubes, that the large bubble was split up. (See photographs 12 to 16.)

In the commencing stages of this condition one will often see, when an egg is placed over the candle, a very large and regular or irregular air space. On rotating such an egg a trembling of the membrane enclosing the albumin is noted. No typical watery white movable bubble is seen. If such an egg is shaken lightly, movement is heard, and if a more vigorous shake be given, one hears a sudden flop, and on recandling one observes the typical bubble of the watery white egg.

This one would interpret as the early stages in the formation of an egg that may be a watery white egg. On carefully cracking and examining an egg that shows on being candled, this trembling of the air space one finds the membrane lining the shell intact, but the membrane enclosing the albumin is not tense and stretched from side to side showing a well-defined air space as in a normal egg, but is relaxed or partially collapsed and may be actually resting on the albumin.

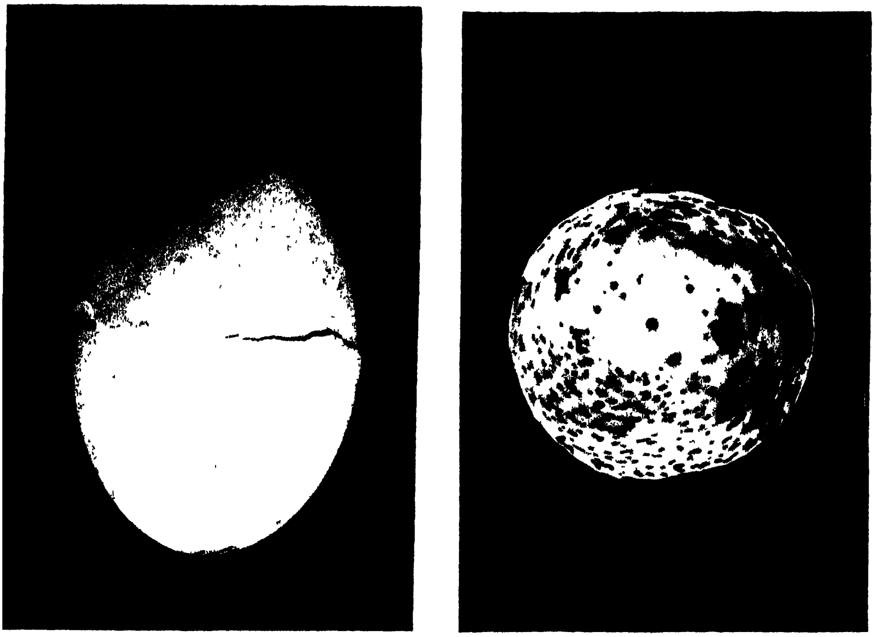


Figure 3. Figure 4.

Fig. 3. Outer surface of egg shell after being immersed in stain.
Fig. 4. Inner surface of same shell showing areas that have taken up stain.

Jordaan extracted in a Weekly Press Service to the South African farmers on 6th September, 1932, a preliminary investigation by Halnan, E. T., of the Animal Nutrition Institution, Cambridge, on watery white eggs. His views were that two types of watery white eggs were present and describing what undoubtedly is the commencing stage of such an egg as a definite type. Our observations on this subject were made as far back as the beginning of 1932.

Candling of Watery White Eggs.

This is the method in vogue among graders in the large egg circles and wholesale businesses. Any egg that shows the movable air space or the tremulous membrane is classed as a defective egg. Handling large numbers of eggs as these firms do this is the only test they can carry out, and yet many eggs are classified as watery white eggs which are decidedly not weak albumin eggs. Further, many eggs which on candling are passed as normal eggs have decidedly watery albumin and should be discarded. Proofs of these statements will be given farther on. The only conclusions one can draw are that one cannot rely on candling alone for recognizing this condition and yet what other method can be used for testing intact eggs.

(*b*) The Shells.

Texture of Shells.

During the examination of many eggs by means of the candle, one's attention is often drawn to the fact that many of these eggs have shells which are classed by poultrymen and egg-traders as markedly porous shells. Instead of the light coming through evenly as in a so-called good shell, it shows numerous small areas of bright light. (See figures 1 and 2.)

In other eggs the light of the " candle " comes through very distinctly, making it very evident that such shells are weak shells. A number of such shells show very fine cracks, which are not very evident away from the " candle ".

Another group of shells will, over the candle, appear as good, normal shells.

When one examines eggs that have been tested and given as normal eggs one will just as frequently meet with all these changes described above, so that one is forced to the conclusion that if a watery white egg has such a supposedly porous shell it is simply a coincidence. It was decided to test out many of these shells for the so-called excessive porosity. Weston, W. A. R. D., and Halnan, E. T. (1927), working on Black Spot in eggs, described a method of staining the pores with starch solution and alcoholic solution of iodine. Hays, F. A., and Sumbardo, A. H. (1927), in an article on shell pore studies, describe another method of staining that consist of the use of alcoholic eosin and allowing exposure to this for about six hours.

Both these methods were tried out with varying success. In addition to eosin other stains were used such as methylene blue (aqueous and alcoholic), alcoholic neutral red, rose aniline violet, May Grünwald and Giemsa. Of all these stains the ones giving the best results were May Grünwald and Giemsa. Two methods were adopted: (*a*) breaking of shell and staining one half by filling with stain and the other half was allowed to float in the stain—staining usually took place in about 10 minutes: (*b*) immersing of intact egg before being broken in the stain. Here again staining usually took place within 10 minutes.

As a result of the first method it was found that the majority of eggs in which the stain was placed in the shell showed very many pin-point areas of stain, some very numerous and in some of these many appeared quicker than others. Shells from both normal and " watery white " eggs were tested. Results showed that both groups of shells stained almost similarly, and the differences between them were negligible. When we came to those egg-shells that were floated on the stain quite another picture was observed. Some eggs showed nothing on the outside when the shell was washed, and yet inside very large numbers of small areas of stain or a few large areas of stain were present. In view of this one decided to immerse all eggs for from 3 to 5 minutes in undiluted stain.

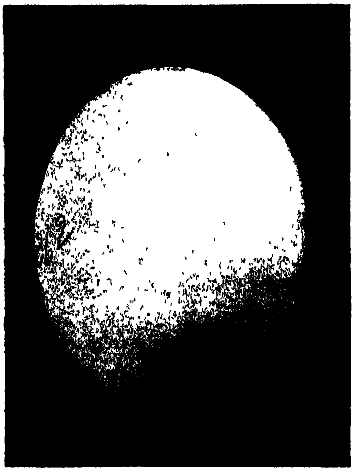


Figure 5.

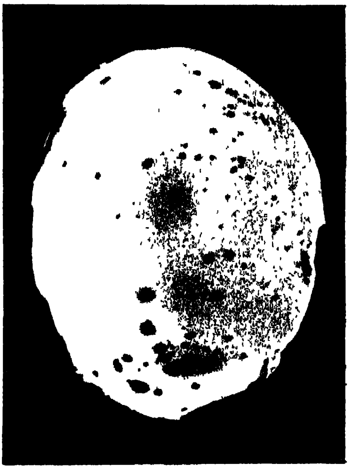


Figure 6.

Fig. 5. Outer surface of egg shell after being immersed in stain.
Fig. 6. Inner surface of same egg showing stained areas.

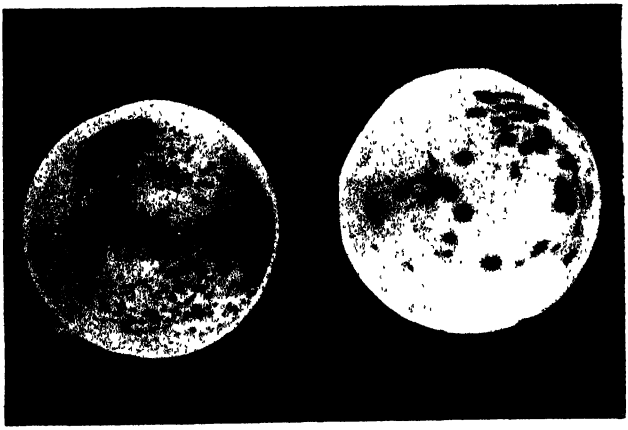


Fig. 7. Internal surface of same egg shell showing stained areas, outer surface showed nothing.

Normal Eggs.—Eggs from the same hens examined within 12 hours of laying day after day for almost two months show sometimes a very few pin-point areas of stain, otherwise nothing; on some days they are perhaps more numerous than others. When these eggs are kept any time over a week at room temperature and stained these pin-point stained areas are more numerous. This appears to indicate a form of drying out of the shell.

Watery White Eggs (Candle Test).—Such rejected eggs were sent me from the Natal egg circle at Durban. Many of these showed very numerous pin-point stained areas, more so in very many cases than did old normal eggs. Others again showed very numerous large areas. So much stain was in some cases taken in that the albumin had a slight bluish tinge. These eggs, even after immersion for 10 to 15 minutes in undiluted Giemsa, when washed showed hardly a sign of the stain as compared with normal shelled eggs that were usually pink or bluish in colour. Such eggs were frequently thin shelled eggs, and one had to be careful even when handling them in case they cracked. Many of these were rough and lustreless shells as compared with the usual smooth and glossy shell of the normal egg. That they were definite "watery white" eggs was shown by measuring the albumin. To give only two examples:—

Egg No. 128 showed 19·5 c.cs. thin albumin and 8 c.cs. thick albumin.

Egg No. 126 showed 19 c.cs. thin albumin and 3 c.cs. thick albumin.

Photographs showing the interior of the shell stained were taken.

Porosity.

It is claimed by many big poultrymen that porous shelled eggs have a low degree of hatchability. When asked to describe what they mean by a porous shelled egg they describe the condition shown in Fig. 1. When such eggs are taken and stained this suggested porosity is more often than not absent. Writers refer to visibly porous eggs but refrain from saying how they know these eggs are porous. The group of eggs that should be classed as porous are those shown in photographs 3 to 8, and yet, over the "candle" before immersion in stain, they did not approach the eggs seen in photograph 1 as far as so-called porosity was concerned. One can understand thin fragile shelled eggs being classed as porous, but the majority of so-called porous eggs are usually firm and strong. One would say that there was an irregular deposition of lime in the shell rather than that the shells were porous. The impression that these eggs are porous is probably due to the fact that people think the light areas in the shell over the candle are actual pores. It is certain, however, that the greater amount of porosity in eggs as judged by the stained shells is to be found in definite cases of watery white eggs.

Membrane.

As is well known, when an egg is laid no air space is seen if the egg is "candled" immediately. Examined a day later, there is a distinct small air space. Every day after being laid the air space gets greater as the contents of the egg become less due to evaporation through the shell. This takes the form of a shrinking of the membrane.

The larger the air space the greater the surface area of albumin lining membrane which is detached from the shell membrane. The surface area being increased and the support from the shell being removed the membrane has to bear the whole of the shell contents. Weston, W. A. R. D., and Halnan, E. T. (1927), quoting Lillie, state " The shell membrane consists of two layers, a thick outer layer next to the shell and a thinner one next the albumin. Both are composed of matted organic fibres (more delicate in the inner than in the outer layer) crossing one another in all directions ". It will thus be seen that the membrane enclosing the albumin will probably rupture easier than the membrane lining the shell. Hays, F. A., and

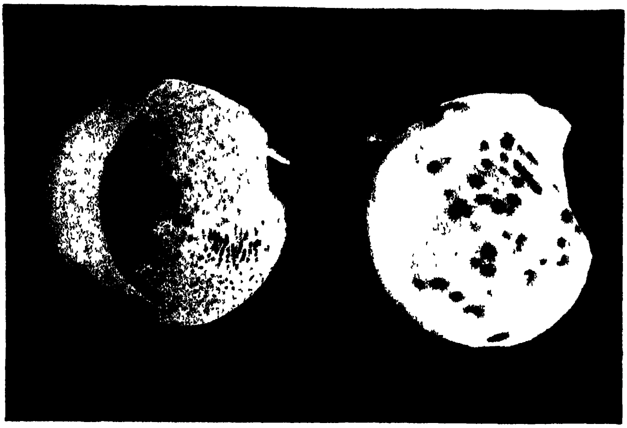


Fig. 8. Internal surface of same egg shell showing stained areas, outer surface showed nothing.

Fig. 9. Empty test tube.

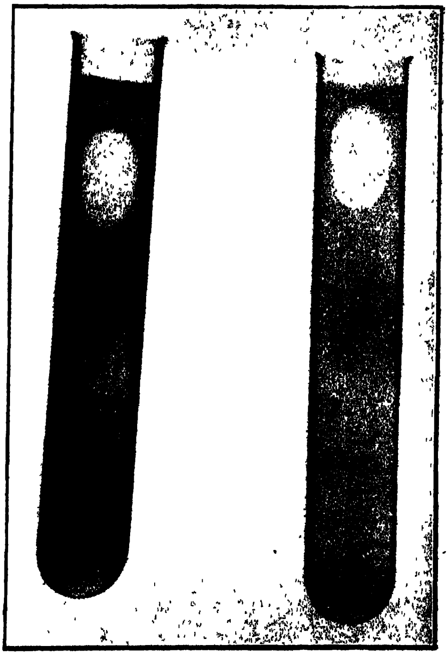


Fig. 10. Thin albumin (before shaking).

Fig. 9. Empty test tube.
Fig. 10. Thin albumin (before shaking).

Sumbardo, A. H. (1927), state that the numbers of pores per sq. mm. was greater in the inner shell membrane than in the outer shell membrane in all cases. Again this bears out the views of the first two workers. This air space is used as a guide by graders to tell the age of the egg.

In the case of " watery white " eggs as determined by candling one observed that no air space was present. This was due to the inner membrane having been ruptured.

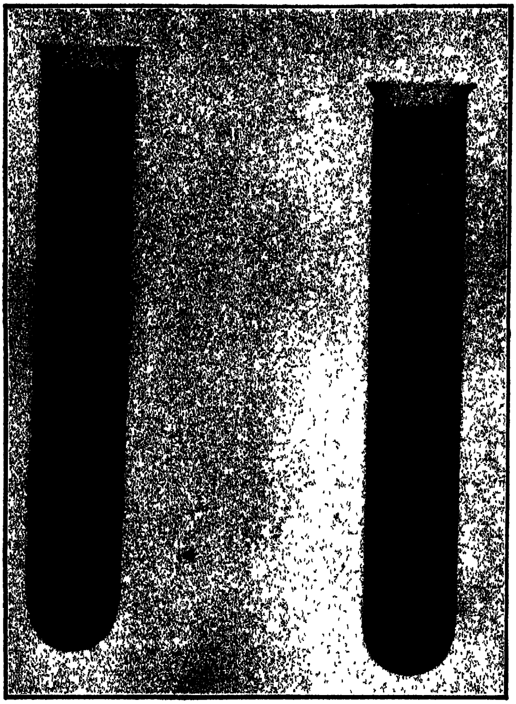


Fig. 11. Thick albumin (before shaking).

Weight.

There was no more variation in the weight of candle-determined watery white eggs than in normal eggs. Graphs made of normal eggs laid daily and weighed within 2 hours of being laid showed marked variations day by day in their weights. As has been noted previously by other workers the heavy egg, in almost every case, is followed by a lighter egg. One even lighter may be laid, and then once more the rise to another heavy egg.

It was impossible to obtain a continuous supply of eggs from any one fowl whose eggs showed a candled watery white, as these eggs come from all over Natal and are not marked as from any individual fowl. However, in the light of the whole egg weight of watery white egg resembling closely normal eggs one would surmise that here also the shell weights of watery white eggs were also fairly constant.

When one comes to the precentage comparison of weight of shell to weight of egg the following results are seen:—

Hen No.	Number of Eggs Laid.	Percentage of Shell to Egg Weight.		Percentage of Shell Weight to Egg Weight (Average).
		Maximum.	Minimum.	
		%	%	%
324............	27	10·8	7·4	8·9
399............	32	12·6	10·3	11·3
342............	21	13·3	8·9	10·5
947............	33	12·6	8·1	9·9
362............	31	13·6	9·7	11·6
294............	31	11·6	8·6	9·9
397............	31	13	9	10
354............	27	12·4	9·2	10·6
	233	Av. 12·4	Av. 8·9	Av. 10·3

These figures for normal eggs show that for 233 eggs the average percentage of shell weight to whole egg is 10·3 per cent. The figures for 92 candled watery white eggs showed an average percentage of shell weight to whole egg weight of 9·9 per cent. with a maximum percentage weight of 12·1 per cent. and a minimum percentage weight of 9·7 per cent.

It would appear as if there is a slight difference between percentage weight of shell to whole egg between normal eggs and candled watery white eggs.

(c) The Albumin.

(a) *Technique of Measuring the Amount of Albumin.*

A funnel, a 50 c.cs. measuring cylinder, and a sieve with meshes 9 to 1 inch were all that was needed for measuring the thin and the thick albumin. The egg was cracked and the albumin allowed to run into the sieve, the thin portion ran through until no more would pass the meshes. This amount was then read off in c.cs., the thick albumin was then poured into the cylinder and again the reading taken. In this way the amounts of thin and thick albumin could be estimated.

(b) *Quantities of Thin and Thick Albumin in Candled Normal Eggs taken shortly after being Laid.*

It is well known that the albumin in the normal egg is made up of both thin and thick albumin, the thick being usually in excess of the thin albumin. It has been stated that thin albumin may be present in normal eggs varying from 18 per cent. to 53 per cent. and the egg is still classed as normal. From this work after examining a large number of eggs one did not meet with so high a percentage as 53 per cent., our highest figure was 51·8 per cent. and our lowest 25·4 per cent. The percentages of thick albumin were from 48·2 per cent. to 74·6 per cent. (See tables.)

Holst, W. F., and Almquist, H. J. (1931), give a table showing the percentage of thick white from eggs of different fowls. The variations in the percentages are small for each individual hen and the greatest difference is 8 per cent. From our figures taken over long periods for the eggs of various hens much greater variations are shown.

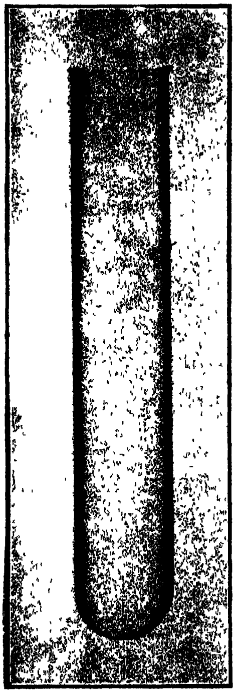


Fig. 9a. Empty test tube.

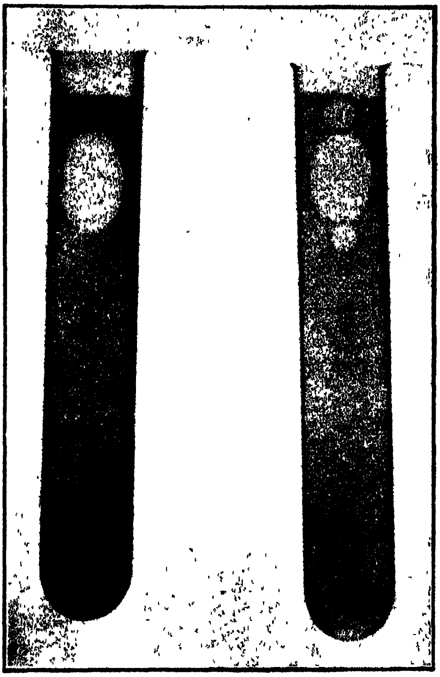


Fig. 10a. Thin albumin (after shaking).

Fig. 9a. Empty test tube.
Fig. 10a. Thin albumin (after shaking). Note bubbles as compared with before shaking.

Hen 294: Maximum difference between lowest and highest percentage, 17·9 per cent.
Hen 397: Maximum difference between lowest and highest percentage, 15·4 per cent.
Hen 362: Maximum difference between lowest and highest percentage, 20·4 per cent.
Hen 342: Maximum difference between lowest and highest percentage, 18·9 per cent.
Hen 947: Maximum difference between lowest and highest percentage, 12·2 per cent.
Hen 324: Maximum difference between lowest and highest percentage, 13·5 per cent.
Hen 399: Maximum difference between lowest and highest percentage, 21·5 per cent.
Hen 354: Maximum difference between lowest and highest percentage, 13·6 per cent.

(c) *Quantities of Thin and Thick Albumin in Candled Watery White Eggs.*

When the albumin of these eggs is measured remarkable differences are seen. Many of these eggs give measurements which correspond with the measurements of albumin of normal eggs. The smallest percentage of thin white was 18·1 per cent., while the smallest thick white was 6·3 per cent. The largest percentage of thin white was 93·7 per cent., while that of thick white was 81·9 per cent.

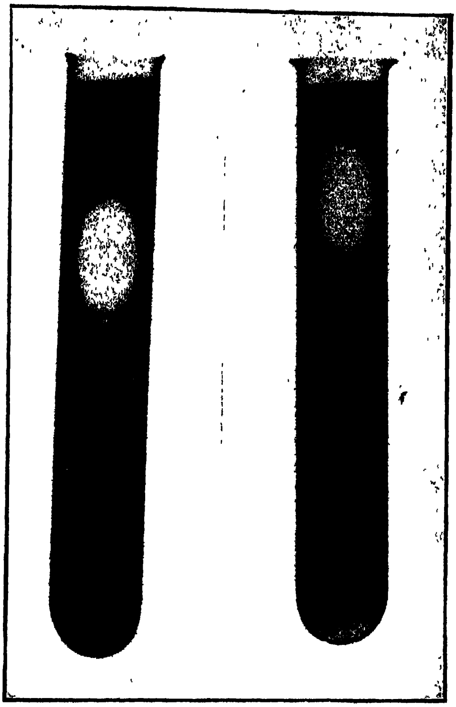


Fig. 11a. Thick albumin (after shaking).

(d) *Quantities of Thin and Thick Albumin in Candled Normal Eggs at Varying Intervals after being Laid.*

The eggs used in this experiment were those taken from the groups of fowls whose eggs had been tested and examined within 24 hours of being laid. They were kept at room temperature in a single layer in a cardboard egg container for varying periods of days. The temperature was usually between 70° and 75°. Before measuring the albumin, all were tested over the candle and were definitely not watery white eggs. Several were shaken and then examined and we found the typical watery white appearance over the candle. The figures from these birds are interesting. The maximum and minimum percentages of both thin and thick albumin from these eggs will be given. In the tables will be found the details of the eggs examined of each fowl. The maximum percentage of thin albumin was 87·3 per cent., while that of thick albumin was 68·1 per cent. The minimum percentage of thin white was 31·9 per cent., while that of thick white was 12·7 per cent.

From the graphs it will be seen that as a result of being kept the total amount of albumin in these eggs was diminished.

(e) *Reaction of Albumin of Normal and Defective Eggs.*

Sharp & Powell (1927) state that the pH of the whites of freshly laid eggs is about 7·6 and that after a few days in a well-ventilated room it will reach 9·5, provided they are not oil-dipped, or placed in water or water-glass solution. They further state that untreated eggs kept in a badly ventilated room (full of eggs) may have a pH considerably lower than 9.5. They further quote Sharp and Whitaker and Stark and Sharp, who claim it is possible for organisms to grow in media with a pH of 7·6 or slightly higher, but never if the pH be 9·5.

We found here that the pH of normal eggs, some tested just after being laid, other a day or more old, varied from 6·8 to 8·5. We then proceeded to test the albumin of naturally and artificially caused watery white eggs. Very little difference was found, the pH varying from 7·2 to 8.

(d) DISCUSSION.

Armed with the figures obtained about thin and thick albumin from normal eggs recently laid and from normal eggs stored we are now in a position to give a better and more exact definition of a watery white egg. The candle appearance of the moving air space is not necessarily an indication of a watery white egg. Again, the absence of the movable air space is no indication that the egg is a normal egg or a watery white egg. Thus we are forced to the conclusion that candling is not a reliable test for seeing whether an egg is a watery white one or not; however, in the circumstances it is the only one available, and although many eggs are rightly condemned many others as far as their albumin is concerned are normal.

The appearance of the shell as seen over the candle is no indication as to its porosity except in the case of a shell that shows the light clearly and brightly through it. This indicates a thin shelled egg. A watery white egg may have a good shell or the poultry-man's so-called porous shell or a thin shell, conditions which may also be found in normal eggs. Many true watery white eggs show on being stained this excessive porosity from the outside to the inside, this condition has not as yet been seen in a normal egg. Such egg shells prior to being stained, when examined over the candle show nothing abnormal.

The shells of true watery white eggs are in at least 90 per cent. of cases whitish in colour; only rarely are brown eggs subject to this condition.

The membrane of candled watery white eggs was invariably ruptured; this is borne out by the X-ray plates. In one of the plates a partial detachment of the membrane is shown. This is the tremulous condition mentioned by Halnan.

There does not seem to be much difference between the percentage weight of shell as compared with total weight of eggs of normal and watery white eggs.

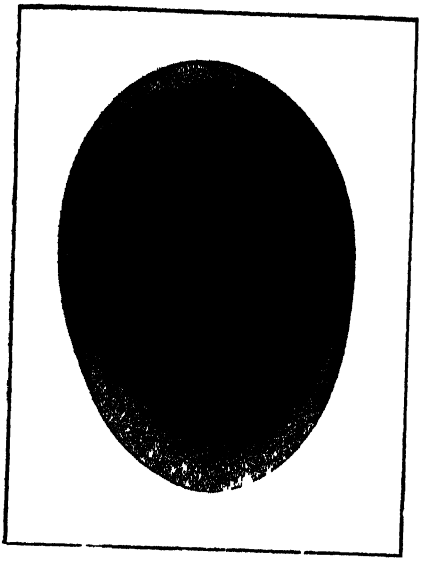


Fig. 12

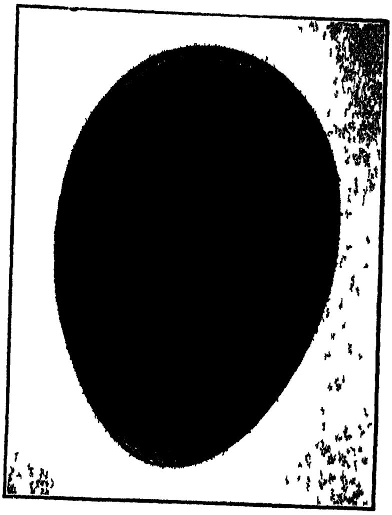


Fig. 13.

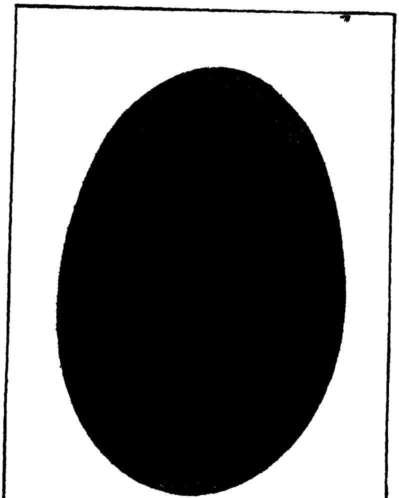


Fig. 14

Figs. 12, 13, 14. X-ray of candled watery white egg showing air bubbles but no air spaces.

From observations made it appears that the keeping qualities, so far as thick and thin albumin goes, of normal eggs vary considerably. There is a decided tendency towards an excess of thin albumin over thick albumin. This commencing change started from about the 7th day and became marked from the 10th to 14th days. Over the candle one was able to see a large air space indicating an old egg. By giving such eggs a slight shake a flop would be heard, and on recandling one would obtain the typical candled watery white egg appearance. This would rather point to the fact that many eggs sent overseas, the trip lasting at least 16 to 21 days, from the time of being laid, are likely to be watery white eggs on their arrival at their destination without in many cases showing it over the candle.

EXPERIMENTAL PRODUCTION OF WATERY WHITE EGGS.

We found that fresh eggs kept at room temperature for fourteen days and shaken gave the typical watery white appearance on being candled; when their albumin was measured this was confirmed. Prior to being shaken, these were candled and conformed to the graders' opinion that they were normal, although not new laid.

We next collected eggs immediately on being laid and placed them in a bacterial incubator running at 37° C., which corresponded to a temperature of 98·6° F. Such a temperature would be considered a fair one in comparison with what prevails in Natal during the time this condition of defective eggs is most common. At definite intervals these eggs were taken out and examined over the candle, and apart from showing a larger air space than would eggs which had been kept at room temperature for the same period, they were normal. On giving these a shake we were again able to set up the candled watery white egg appearance. The shortest time in which we were able to produce this appearance was in two cases, 24 hours. Unfortunately at that time we did not measure the albumin, so we are unable to state whether or not these were true watery white eggs. (See Table 1.)

SUGGESTED CAUSES.

One would first consider those eggs whose appearance over the candle suggests a watery white condition and yet on measuring the albumin they appear quite normal.

(*a*) WARMTH.

The points in favour of warmth are:—

1. The fact that the largest number of affected eggs is found during the warmest months of the year, and the egg export season is usually from mid-September to about the end of January.

2. The frequent placing of nests for laying in such a position that the sun heats them.

3 The fact of having too few nest boxes and eggs being collected not frequently enough, with the result that the earliest laid eggs have almost 10 hours' incubation.

4. Eggs are frequently not cooled after being laid, but are placed straight into egg boxes or stored in warm houses without any moisture in them. That this is a most important point is borne out by the advice given to poultry-men overseas, viz.: "Get a layer of fresh grass; upon this place a layer of hay and lay the eggs on their sides on it. A temperature of 50° F. is the best one for storing eggs".

5. The fact that eggs that are kept at 37° C. for 24 hours and then shaken show changes indistinguishable from watery white egg changes.

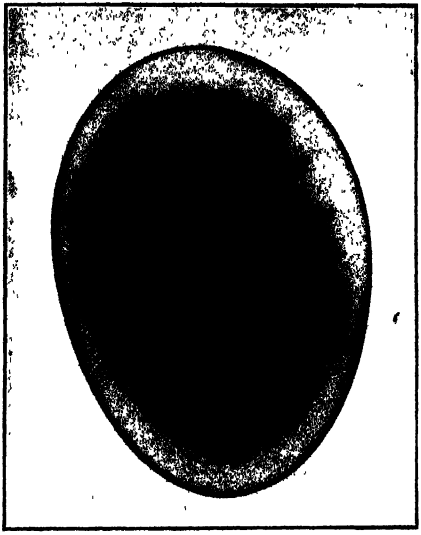


Fig. 15. X-ray of candled watery white egg showing detachment of inner shell membrane. No air bubbles present.

6. In all cases of these incubated eggs, one observes a fairly large air space, which is an indication of drying out. In eggs kept at room temperatures, especially in cool rooms, this air space increases day by day, but not so rapidly as when eggs are subjected to outside temperatures. The larger the air space, the more room for the albumin and yolk to move, with a consequent detachment of the albumin membrane from that lining the egg shell and its subsequent rupture.

(*b*) VIBRATION OR MOVEMENT.

The points in favour of vibration are:—

1. Eggs heated at 37° C. for 24 hours, when shaken, take on a similar appearance to the affected eggs under discussion.

2. The majority of naturally caused watery white eggs have come to the egg circle from a distance which has entailed travelling. In transit they have been subjected to much shaking and knocking about from the time they have been loaded on the farm to the time they have been unloaded at the receiving depot.

3. In the case of eggs being found affected on arrival overseas, in spite of being passed as normal when packed in South Africa, the same factors are present, viz., handling, repacking, cartage from depot to dock, loading, vibration, however slight, for 16 to 21 days continuously from the ship's engines, pitching and rolling of the ship, and finally unloading and dispatching to various egg distributing centres.

When one comes to consider the possible cases of true watery white eggs the following conclusions are reached. The age of the egg possibly plays an important part. In no candled affected egg is there any air space for the graders to say whether an egg is old or not, but one frequently meets with " stuck yolks ", which is an indication of age. On breaking affected eggs one frequently finds degenerative changes in the yolk, such changes also being present in known old eggs.

Many eggs which have been kept and are apparently normal over the candle are distinctly watery white eggs when measured; this indicates that the candle appearance is only secondary to the true cause. From the graphs it will be seen that these changes take place the longer the egg has been kept. This has been recognized by St. John, J. L., and Flor, I. H. (1931), who state " there were comparatively few number one eggs and none after the seventh day of storage ". The change from thick to thin white is probably due to some extent to the temperature at which they are stored and probably due to enzyme reaction converting the thick to thin white.

So far we have not observed an egg being laid as a watery white egg.

With such eggs shaking or vibration next plays a part. This was actually utilized by Platt, C. S. (1929), to identify watery white eggs. He states " the case was then shuffled back and forth across the floor moving it at arms' length each time. One hundred movements were used in each instance. This had been previously found necessary for the proper determination of the ' watery white ' eggs ".

We now come to the possibility of porous shells playing a part. One refers to those shells that take stain excessively as shown in the photographs and those shells which are definitely weak, brittle, and thinner than a normal shell. Excessive evaporation must take place. It was noted in our work that many such thin shelled eggs contained very small amounts of thick white, e.g., 3 c.cs. as compared with 25 c.cs. of thin white, 2·5 c.cs. as compared with 20 c.cs., and 1·5 c.cs. as compared with 22·5 c.cs. to quote three examples. The causation of such thin shelled eggs is referred to by Taylor, L. W., and Martin, J. H. (1928), whose summary states three main causes: (1) wrong feeding including vitamine and calcium deficiency, (2) the inherited inability to produce heavy shelled eggs, and (3) pathological conditions in the oviduct.

Finally one would suggest that towards the end of an egg-laying period among hens that lay over 200 eggs a season there is likely to be a big strain on her and it is possible that some portion of the oviduct may in some way be temporarily affected. This would only occur among a limited number of birds and is not likely to be a very common cause.

We are unable to make any definite statement on the possibility of porous shells playing a part in the causation of watery white eggs.

SUMMARY.

(*a*) The method of testing eggs by means of the candle is not an exact method for showing whether an egg is a watery white egg or not.

(*b*) It is reliable for showing whether or not the albumin lining membrane is ruptured or not.

(*c*) Under the present conditions it is, however, the only method that can be used.

(*d*) All eggs showing air bubbles or a bubble are not watery white eggs.

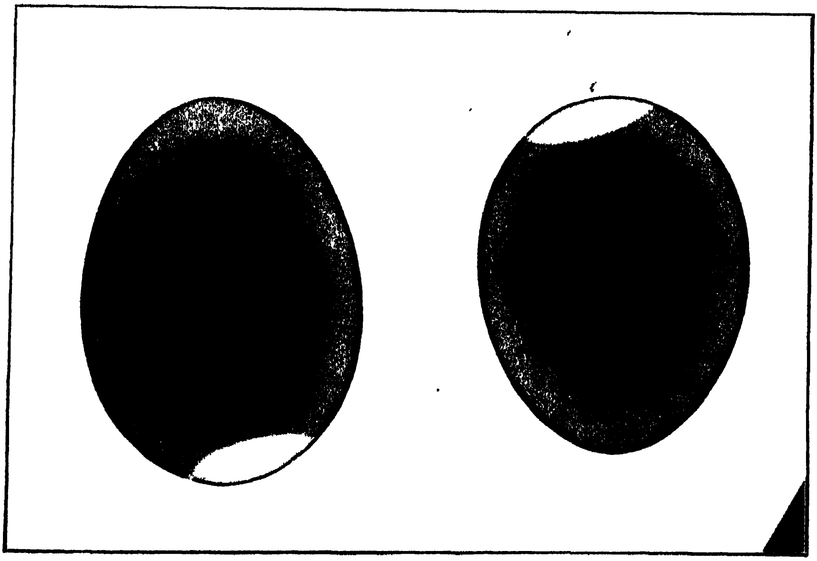


Fig. 16. X-ray of two normal eggs showing air spaces.

(*e*) Many eggs not showing air bubbles or a bubble are definitely watery white eggs.

(*f*) The poultryman's porous shelled egg as distinct from a thin brittle shelled egg is in the majority of cases decidedly not porous.

(*g*) Many shells which on being tested by stain are markedly porous, are, when placed over the candle prior to staining, apparently normal shells.

(*h*) The longer an egg is kept the more porous the shell becomes.

(*i*) Porosity does not seem to play an important part in the causation of watery white eggs.

(*j*) In all eggs showing air bubbles or a bubble the inner shell membrane covering the albumin is ruptured.

(*k*) There is not much variation in the percentage of shell weight to total egg weight between normal and watery white eggs.

(*l*) Definite watery white eggs showed a maximum percentage of thin albumin of 93·7 per cent.

(*m*) Normal eggs kept for varying intervals became in many cases definite watery white eggs although this could not be shown over the " candle ".

(*n*) The reaction of the albumin of normal and watery white eggs was practically similar.

(*o*) There are probably a number of causes all acting together or at times separately to set up watery white eggs.

(*p*) These are probably warmth, vibration or movement, storage or age of eggs, excessive porosity in a few cases, and probably the result of strain in hens towards the close of a heavy egg-laying period in a further number of cases.

ACKNOWLEDGMENTS.

I would like to thank Mr. L. F. Forsyth, of Maritzburg, for very kindly supplying me daily with large numbers of eggs from definite trap-nested hens. To Mr. Slatter, Secretary of the Durban depot of the Natal Co-operative Egg Circle, my thanks are due for kindly sending me all the watery white eggs I worked on. I must also acknowledge advice and suggestions so readily given me by many practical poultrymen of Natal. To Dr. Grieve, of Maritzburg, my thanks are due for taking the X-ray plates of affected eggs, and to Mr. Hill, my assistant, for the photographs and willing help.

APPENDIX 1.

NORMAL EGGS.

Date.	No.	Weight of Egg.	Weight of Shell.	Percentage of Shell to Egg. Weight.	Thin Albumin.	Thick Albumin.	Total Albumin.	Percentage Thin to Thick Albumin·
		gms.	gms.	%	c.cs.	c.cs.	c.cs.	% %
22.10.32	294	57·78	6·6	11·4	9	17·5	26·5	33·9–66·1
24.10.32	,,	64·33	7·1	11	13	17	30	43·3 56·7
25.10.32	,,	58·21	6·3	10·9	9	16·5	25·5	35·2–64·8
27.10.32	,,	58·03	6·2	10·7	9	19	28	32·1–67·9
28.10.32	,,	56·21	5·68	10·1	8	17·5	25·5	31·6–68·4
29.70.32	,,	56·59	6·55	11·5	10·5	18·5	29	36·2–63·8
31.10.32	,,	61·36	6·97	11·3	11	19·5	30·5	36 64
1.11.32	,,	58·65	5·57	9·5	8·5	19	27·5	30·9–69·1
2.11.32	..	57·75	5·72	9·9	11·5	16·5	28	41 –59
4.11.32	,,	61·80	5·69	9·2	10·5	19·5	30	35 –65
5.11.32	..	56·95	5·66	9·9	10	16	26	38·4–61·6
7.11.32	..	63·11	6·31	10	9·5	21·5	31	30·6 69·4
8.11.32	,,	56·72	5·58	9·8	8·5	18	26·5	32 –68
9.11.32	..	55·60	6·46	11·6	9	16·5	25·5	35·2–64·8
11.11.32	..	60·25	5·88	9·7	11	19	30	36·6 63·4
13.11.32	,,	57·13	5·92	10·3	11	16,	27	40·7–59·3
14.11.32	..	63·62	5·66	8·9	10·5	21·5	32	32·8 67·2
15.11.32	,,	57·02	6·20	10·8	6·5	19	25·5	25·4–74·6
17.11.32	..	63·77	6·08	9·5	10·5	21	31·5	33·3–66·7
18.11.32	,,	57·43	5·51	9·6	8·5	18·5	27	31·4 68·6
19.11.32	..	59·58	6·18	10·3	12	16·5	28·5	42·1–57·9
21.11.32	,,	62·50	5·93	9·4	12	18	30	40 –60
23.11.32	..	59·88	5·61	9·3	8	20	28	28·5–71·5
25.11.32	..	65·84	5·70	8·6	11·5	20·5	32	35·9–64·1
27.11.32	..	61·11	5·86	9·5	10·5	18·5	29	36·2–63·8
28.11.32	..	67·54	6·40	9·4	11	21·5	32·5	33·8–66·2
29.11.32	,,	55·39	5·54	10	10	16	26	38·4–61·6
1.12.32	..	61·52	5·66	9·2	11·5	19	30·5	37·7–62·3
2.12.32	,,	57·61	5·79	10	10·5	18·5	29	36·2–63·8
4.12.32	..	64·33	5·59	8·6	9·5	23·5	33	28·7–71·3
5.12.32	,,	57·98	5·40	9·3	11	16	27	40·7 59·3
22.10.32	399	62·79	7·76	12·2	12·2	12·8	25	48·8–51·2
23.10.32	,,	61·75	7·65	12·3	12	15·5	27·5	44·1–55·9
25.10.32	,,	64·10	7·32	11·4	10	20	30	33·3–66·7
26.10.32	,,	61·97	7·33	11·8	12	17	29	41·3 58·7
27.10.32	,,	61·27	7·48	12·2	11	17	28	39·2 60·8
29.10.32	,,	64·01	7·63	11·9	13	12·8	25·8	50·3–49·7
30.10.32	,,	61·73	7·71	12·4	11·5	17·5	29	39·6–60·4
31·10·32	..	60·62	7·41	12·2	11	17	28·5	38·5–61·5
2.11.32	..	65·22	7·26	11·1	13	18	31	41·9–58·1
3.11.32	..	61·79	6·69	10·8	12·5	17·5	30	41·6–58·4
4.11.32	,,	60·48	6·66	11	10·8	16·7	27·5	39·2–60·8
6.11.32	..	63·76	6·83	10·7	13	16·5	29·5	44 –56
7.11.32	..	62·18	7·11	11·4	11	17	28·	39·2–60·8
9.11.32	,,	64·88	7·24	11·1	11	19·5	30·5	36 –64
10.11.32	,,	62·14	7·16	11·5	11	17·5	28·5	38·5–61·5
11.11.32	,,	61·65	7·14	11·5	10	18·5	28·5	35 –65
13.11.32	,,	63·31	7·00	11	9·5	20	29·5	32·3–67·7
14.11.32	,,	63·45	7·00	11	12	18	30	40 –60
15.11.32	.,	61·62	7·50	10·5	11·5	17·5	29	39·6–60·4
17.11.32	,,	63·51	7·26	11·4	11·5	17·5	29	39·6–60·4

Date.	No.	Weight of Egg.	Weight of Shell.	Percentage of Shell to Egg. Weight.	Thin Albumin.	Thick Albumin.	Total Albumin.	Percentage Thin to Thick Albumin.
		gms.	gms.	%	c.cs.	c.cs.	c.cs.	% %
18.11.32	399	60·67	6·50	10·7	11·5	16	27·5	41·8–58·2
19.11.32	„	61·60	7·22	11·7	12·5	15·5	28	44·6–55·4
21.11.32	„	63·40	7·25	11·4	13	17	30	43·3–56·7
22.11.32	„	62·56	6·71	10·7	13	16 5	29·5	44 –56
24.11.32	„	59·35	6·68	11·2	12	15	27	44·4–55·6
25.11.32	„	61·92	6·39	10·3	13	16	29	44·8–55·2
28.11.32	„	61·57	6·31	12·6	10	19	29	34·4–65·6
29.11.32	„	60·35	6·82	11·3	12	16	28	42·8–57·2
1.12.32	„	61·19	6·54	10·7	8·5	21	29·5	28·8–71·2
2.12.32	„	61·10	6·76	11	10	18·5	28·5	35 –65
4.12.32	„	60·41	6·69	11	12	16	28	42·8–57·2
5.12.32	„	63·19	7·09	11·2	12·5	16·5	29	43·1–56·9
22.10.32	362	56·60	6·9	10·4	10	15	25	40 –60
23.10.32	„	54·29	7·3	13·5	7	15	22	31·8–68·2
25.10.32	„	58·55	7·5	12·9	9·5	15·5	25	38 –62
26.10.32	„	57·68	7·8	13·6	11	15	26	42·3–57·7
28.10.32	„	60·91	6·55	10·7	12	14	25	48 –52
29.10.32	„	58·10	7·39	12·7	10·5	15·5	26	40·3–59·7
31.10.32	„	60·64	7·71	12·7	9	19·5	28 5	31·5–68·5
1.11.32	„	56·85	6·38	11·2	11·5	14	25·5	45 –55
2.11.32	„	55·52	6·67	12	11	14	25	44 –56
4.11.32	„	59·47	6·27	10·5	13	14·5	27·5	47·2–52·8
5.11.32	„	57·45	6·81	11·8	11	14	25	44 –56
7.11.32	„	59·18	6·22	10·5	10	16·5	26·5	37·7–62·3
8.11.32	„	57·32	6·54	11·4	10·5	14·5	25	42 –58
10.11.32	„	59·45	7·00	11·7	8·5	18·5	27	31·4–68·6
11.11.32	„	57·45	6·39	11·1	11·5	14·5	26	44·2–55·8
13.11.32	„	57·86	7·08	12·2	11	15·5	26·5	41·5–58·5
14.11.32	„	57·39	7·31	12·7	11·5	14	25·5	45 –55
15.11.32	„	55·94	7·48	13·3	10·5	14·5	25	42 –58
17.11.32	„	57·93	6·92	11·9	11·5	14	25·5	41·5–58·5
18.11.32	„	55·56	6·62	11·9	11	15	26	42·3–57·7
19.11.32	„	59·35	6·6	11·1	12·5	15	27·5	45·4–54·6
21.11.32	„	56·05	6·44	11·5	11·5	13·5	25	46 –54
23.11.32	„	58·87	5·73	9·7	13·5	15·5	29	46·5–53·5
26.11.32	„	58·28	6·92	11·8	14	13	27	51·8–48·2
27.11.32	„	57·57	6·06	10·5	12	14	26	46·1–53·9
28.11.32	„	53·82	6·01	11·1	11·5	13·5	25	46 –54
30.11.32	„	57·08	6·25	10·9	11·5	15	26·5	43·3–56·7
1.12.32	„	55·20	5·67	10·2	10·5	14.5	25	42 –58
2.12.32	„	55·12	6·24	11·3	12	13	25	48 –52
4.12.32	„	56·25	6·22	11	11	16	27	40·7–59·3
5.12.32	„	52·58	6·23	11·8	11	14	25	44 –56
22.10.32	342	55·05	7·3	13·3	10	15	25	40 –60
26.10.32	„	55·97	6·5	11·6	8	18	26	30·7–69·3
28.10.32	„	54·55	6·64	12·1	7	15	22	31·8–68·2
30.10.32	„	57·41	7·63	13·2	9·5	16·5	26	36·5–63·5
1.11.32	„	55·75	5·65	10·1	8	17·5	25·5	31·3–68·7
2.11.32	„	57·93	6	10·3	11	16·5	27·5	40 –60

Date.	No.	Weight of Egg.	Weight of Shell.	Percentage of Shell to Egg. Weight.	Thin Albumin.	Thick Albumin.	Total Albumin.	Percentage Thin to Thick Albumin.
		gms.	gms.	%	c.cs.	c.cs.	c.cs.	% %
4.11.32	342	57·94	5·67	9·8	9·5	17·5	27	35·1–64·9
7.11.32	,,	58·9	7·23	12·2	8	19·5	27·5	29 –71
10.11.32	,,	56·82	6·55	11·5	9·5	17	26·5	35·4–64·6
11.11.32	,,	56·76	5·89	10·3	8	17·5	25 5	31·3–68·7
13.11.32	,,	57·02	5·95	10·4	10	17	27	37 –63
14.11.32	,,	60·39	5·9	9·7	12	17	29	41·3–58·7
16.11.32	,,	60·04	6·06	10·1	10·5	18	28·5	36 8–63·2
17.11.32	,,	60·58	5·82	9·6	12	18	30	40 –60
19.11.32	,,	55·72	5·32	9·5	9·5	16	25·5	37·2–62·8
21.11.32	,,	57·41	5·74	10	11	17	28	39·2–60·8
24.11.32	,,	57·37	5·5	9·5	12	15	27	44·4–55·6
27.11.32	,,	58·15	5·13	8·9	9 5	18·5	28	33·9–66·1
1.12.32	,,	52·34	5·43	10·3	6	17·5	23·5	25·5–74·5
4.12.32	,,	59·23	5·60	9·4	10	19	29	34·4–65 6
5.12.32	,,	56 06	5·93	10·5	10·5	15·5	26	40 3–59·7
22.10.32	354	65·525	8·1	12·3	9	18	27	33·3–66·7
25.10.32	,,	63·271	7·9	12·4	7	19	26	26·9–73·1
26.10.32	,,	62·986	7·6	12	—	—	—	—
27.10.32	,,	64·866	7·2	11·1	9	19	28	32 1–67·9
29.10.32	,,	67·26	8·1	12·1	9·5	17·5	27	35·1–64·9
31.10.32	,,	66·29	8·1	12·2	10	19·5	29·5	33·8–66·2
1.11.32	,,	67·06	7·3	10·9	9·5	19·5	29	32·7–67·3
3.11.32	,,	66 24	6·1	9·2	9·5	20	29·5	32 2–67·8
4.11.32	,,	66·12	6·18	9·3	9·5	18	27·5	30·9–69·1
6.11.32	,,	62·71	6·55	10·4	8	17	25	32 –68
5.11.32	,,	66·39	6·32	9·5	10	19	29	34·4–65·6
8.11.32	,,	66·45	7·44	11·2	8·5	20 5	29	29·3–70·7
9.11.32	,,	66·27	7·34	11	9 5	19	28·5	33·3–66·7
10.11.32	,,	66·61	7·37	11	9·5	20	29·5	32·2–67·8
12.11.32	,,	62·69	6·59	10·5	8	18·5	26·5	30 1–69·9
13.11.32	,,	61·78	6·66	10·7	11	16·5	27·5	40 –60
19.11.32	,,	60·18	6·49	10·8	9	18	27	33·3–66·7
21.11.32	,,	63·67	6·7	10·5	8	22	30	26·6–73·4
23.11.32	,,	61·63	6·57	10·6	7	19·5	26·5	26·4–73·6
25.11.32	,,	65·77	6·29	9·5	8·5	20	28·5	29·8–70·2
27.11.32	,,	62·02	6·25	10	9	18·5	27·5	32·7–67·3
28.11.32	,,	63·56	6·04	9·5	8	21	29	27·5–72·5
29.11.32	,,	64·37	6·6	10·2	7·5	19·5	27	27·7–72 3
1·12·32	,,	64·688	6·18	9·5	10·5	17·5	28	37·5–62·5
2.12.32	,,	62·20	6·49	10·4	9·5	18	27·5	30·9–69·1
4.12.32	,,	65·26	6·82	10·4	8·5	21·5	30	28·3–71·7
5.12.32	,,	61·79	6·22	10	9	18	27	33·3–66·7
22.10.32	397	69·05	8·1	11·7	13	21	34	38 2–61·8
24.10.32	,,	66·56	7·3	11	14	18	32	43·7–56·3
25.10.32	,,	60·82	6·7	11	10	19	29	34·4–65·6
27.10.32	,,	63·62	6·9	10·9	11	19	30	36·6–63·4
28.10.32	,,	62·54	6·07	9·7	11	20	31	35·4–64·6
30.10.32	,,	65·05	7·57	11·6	11·5	21·5	33	34·8–65·2
31.10.32	,,	62·46	7·16	13	8·5	21·5	30	28·3–71·7

Date.	No.	Weight of Egg.	Weight of Shell.	Percentage of Shell to Egg Weight.	Thin Albumin.	Thick Albumin.	Total Albumin.	Percentage Thin to Thick Albumin.
		gms.	gms.	%	c.cs·	c.cs.	c.cs.	% %
2.11.32	397	66·70	6·61	9·9	15·5	19·5	35	44·2–55·8
3.11.32	,,	66·10	6·21	9·3	14	18·5	32·5	43 –57
6.11.32	,,	67·99	6·25	9·2	14	20	34	41·1–58·9
7.11.32	,,	66·30	7·30	11	12·5	18·5	31	40·3–59·7
8.11.32	,,	64·02	6·61	10·3	12·5	19	31·5	39·6–60·4
10.11.32	,,	68·35	7·07	10·3	13	21·5	34·5	37·6–62·4
12.11.32	,,	66·38	6·64	10	15	18·5	33·5	44·7–55·3
13.11.32	,,	73·35	7·34	10	14	20·5	34·5	40·5–59·5
15.11.32	,,	64·85	6·54	10	12	21	33	36·3–63·7
16.11.32	,,	63·61	5·94	9·3	9	22	31	29 –71
17.11.32	,,	66·55	6·15	9·2	14	20	34	41·1–58·9
19.11.32	,,	64·72	6·07	9·3	13·5	18·5	32	42·1–57·9
20.11.32	,,	61·86	5·58	9	12·5	18·5	31	40·3 59·7
21.11.32	,,	59·98	5·64	9·4	12	18	30	40–60
23.11.32	,,	64·81	6·11	9·4	13	20·5	33·5	38·8–61·2
24.11.32	,,	62·86	5·75	9·1	11·5	19 5	31	37 –63
25.11.32	,,	62·15	5·83	9·3	10	21	31	32·2–67·8
27.11.32	,,	66·08	6·51	9·8	14	19	33	42·4–57·6
28.11.32	,,	62·50	5·88	9·4	14·5	16	30·5	47·5–52·5
29.11.32	,,	60·72	6·37	10·4	13	17	30	43 3 56 7
1.12.32	,,	68·70	7·23	10·5	17	18	35	48 5–51 5
2.12.32	,,	63·72	6·03	9·4	14	18	32	43 7–56·3
4.12.32	,,	60 52	6·07	10	14	16·5	30·5	45·9–54 1
5.12.32	,,	66·31	6·64	10	14.5	18.5	33	43 9–56·1
22·10·32	324	55·161	5 48	9·9	10·5	15·5	26	40·3–59·7
24.10.32	,,	60·11	6·14	10·2	11·5	18·5	30	38·3–61 7
26.10.32	,,	64·46	5·76	8·9	12	20	32	37·5–62 5
28.10.32	,,	59·80	4·59	7·6	10	20	32	31·2–68 8
29.10.32	,,	57·10	4·71	8·2	10	19	29	34·4–65·6
30.10.32	,,	57·35	5·52	9·6	8·5	20 5	29	29·3–70·7
2.11.32	,,	58·95	4·92	8 3	11·5	18·5	30	38 3–61·7
3.11.32	,,	57·38	4·29	7·4	11	18	29	37·9–62·1
4.11.32	,,	56·80	4·64	8·1	10	15·5	26·5	37·7–62·3
6.11.32	,,	60·23	5·17	8·5	10	19 5	29·5	33·9–66·1
7.11.32	,,	56·35	4·71	7·4	12	16	28	42·8–57·2
9.11.32	,,	59·01	5·80	9·8	9·5	18	27·5	34·5–65·5
11.11.32	,,	57·09	5·45	9·5	8·5	20	28·5	29·8–70·2
15.11.32	,,	58·44	5·24	8·9	9	19·5	28·5	31·5–68·5
16.11.32	,,	55·99	4·85	8·6	10	16·5	26·5	37·7–62·3
18.11.32	,,	60·32	6·08	10·8	11	19·5	30·5	36 –64
19.11.32	,,	59·53	5·52	9·2	11	17	28	39·2–60·8
21.11.32	,,	59·30	5·22	8·8	10·5	18·5	29	36·2–63·8
22.11.32	,,	57·61	4·78	8·2	11·5	16·5	28	41 –59
23.11.32	,,	54·33	5·41	8·9	9	16·5	25·5	35·2–64·8
25.11.32	,,	60·29	5·15	8·5	10·5	19	29·5	35·5–64·5
27.11.32	,,	57·56	4·97	8·6	10	18·5	28·5	35 –65
28.11.32	,,	60·51	6·53	10·7	11	17·5	28·5	38·5–61·5
29.11.32	,,	55·72	4·91	8·8	9	17·5	26·5	33·9–66·1
30.11.32	,,	56·56	5·36	9·2	10	16	26	38·4–61·6
2.12.32	,,	59·41	5·52	9·2	10·5	17	27·5	34·5–65·5
4.12.32	,,	56·60	4·86	8·5	10·5	17	27·5	34·5–65·5

Date.	No.	Weight of Egg.	Weight of Shell.	Percentage of Shell to Egg Weight.	Thin Albumin.	Thick Albumin.	Total Albumin.	Percentage Thin to Thick Albumin.
		gms.	gms.	%	c.cs.	c.cs.	c.cs.	% %
22.10.32	947	52·77	6·66	12 6	9·8	13·2	23	42·6–57·4
24.10.32	,,	52·74	6 41	12·1	9 5	12	21·5	44·1–55·9
25.10.32	,,	54·14	5·74	10·6	9	15·5	24·5	36 7–63 3
26.10.32	,,	53·22	5·46	10·2	8 5	13·5	22	38 6-61·4
28.10.32	,,	56·44	5 68	10	10	15	25	40 –60
29.10.32	,,	54 90	6·18	11·2	10	17	27	37 –63
30.10.32	,,	55·40	6·44	11·6	9	14·5	23 5	38·2 61·8
31.10.32	,,	53 28	5·81	10 9	9	14	23	39 1–60·9
2.11.32	,,	55 63	5 48	9·8	11	13	24	45·8–54·2
3.11.32	,,	54 93	5·07	9 3	10	14·9	24 9	40 1 59·9
4.11.32	,,	54 11	5 36	9·9	11	13	24	45 8–54·2
7.11.32	,,	56·39	6 26	11·1	11·5	13·5	25	46 -54
8.11.32	,,	56·44	5·35	9 4	10	15	25	40 60
9.11.32	,,	54·94	5 45	9·9	10	13 5	23 5	42·5 57 5
10.11.32	,,	54·17	5·45	10	10	13 5	23 5	42 5–57 5
13.11.32	,,	55 67	5·43	9 7	11	14	25	44 56
14.11.32	,,	58 19	6 28	10 8	10	15 5	25 5	39 2–60·8
15.11.32	,,	51 86	4·96	9·5	9 5	14	23·5	40 4–59·6
17.11.32	,,	55 93	5 38	9 6	10·5	14	24 5	42·8-57·2
18.11.32	,,	55·06	5 31	9·6	10	15	25	40 –60
19.11.32	,,	55 18	4 95	9	11	14	25	44 56
20.11.32	,,	53 97	5 46	10 1	10	14 5	24 5	40·8 59·2
21.11.32	,,	52 93	4·92	9 3	11	14	25	44 –56
24.11.32	,,	56·40	5 04	8·9	11	14 5	25·5	43 1–56 9
25.11.32	,,	56 93	4 62	8·1	11	14 5	25 5	43 1–56·9
26.11.32	,,	55 43	4 76	8·5	11 5	12	23·5	48 9–51·1
27.11.32	,,	53·98	5·11	9 6	10	14	24	41·6–58·4
29.11.32	,,	56 09	5·45	9·5	11	14·5	25·5	43·1 56·9
30.11.32	,,	54·49	4·66	8·5	10·5	15·5	26	40·3–59·7
1.12.32	,,	53·22	4·92	9·2	11·5	13	24·5	46·9–53·1
2.12.32	,,	54·55	5·87	10·7	11·5	14 5	26	44 2-55·8
4.12.32	,,	55 42	5·35	9 6	10·5	14·5	25	42 –58
5.12.32	,,	55·54	5·67	10 2	10 5	15	25·5	41·1–58·9

APPENDIX 2.

NORMAL EGGS STORED AND TESTED.

Temperature and Humidity not Constant.

Date.	No.	Date of Test.	Interval.	Thin Albumin.	Thick Albumin.	Total Albumin.	Percentage of Thin to Thick Albumin.
			Days.	c.cs.	c.cs.	c.cs.	% %
6.12.32	947	20.12.32	14	14	6	20	70 –30
8.12.32	„	22.12.32	14	13	8	21	61·9–38·1
9.12.32	„	22.12.32	13	11	10·5	21·5	51·1 –48·9
10.12.32	„	22.12.32	12	12·5	8·5	21	59·5–40·5
11.12.32	„	24.12.32	13	11·5	8·5	20	57·5–42·5
12.12.32	„	29.12.32	17	13	6	19	68·4–31·6
14.12.32	„	6.1.33	23	14	7	21	66·6–33·4
16.12.32	„	6.1.33	21	11.5	9·5	21	54·7–45·3
17.12.32	„	6.1.33	20	10·5	7·5	18	58·3–41·7
20.12.32	„	6.1.33	17	12·5	9	21·5	58·1–41·9
21.12.32	„	6.1.33	16	15	8·5	23 5	63·8–36·2
22.12.32	„	6.1.33	15	11·5	9·5	21	54·7–45·3
23.12.32	„	6.1.33	14	13	8	21	61·9–38·1
25.12.32	„	6.1.33	12	12·5	9	21·5	58·1–41·9
26.12.32	„	6.1.33	11	10·5	12·5	23	45·6–54·4
28.12.32	„	6.1.33	9	11·5	9	20·5	56 –44
1.1.33	„	6.1.33	5	7·5	16	23·5	31·9–68 1
8.12.32	294	21.12.32	14	11	13 5	24·5	44·8–55·2
9.12.32	„	22.12.32	13	15	9·5	24·5	61·2–38 8
14.12.32	„	7.1.33	24	16·5	8	24·5	67·3–32 7
16.12.32	„	7.1.33	22	14	12·5	26·5	52 8–47·2
25.12.32	„	7.1.33	13	13·5	11·5	25	54 –46
30.12.32	„	7.1.33	8	11	13	24	45·8–54·2
6.12.32	324	20.12.32	14	12·5	13·5	26	48·7–51·3
9.12.32	„	23.12.32	14	9·5	17·5	26·5	35·9–64 1
11.12.32	„	24.12.32	13	10	16·5	26·5	37·7–62·3
12.12.32	„	29.12.32	17	13·5	11·5	25	54 –46
16.12.32	„	9.1.33	24	10·5	11·5	22	47·7–52·3
17.12.32	„	9.1.33	23	11·5	10·5	22	52·2–47·8
21.12.32	„	9.1.33	19	15	12	27	55·5–44·5
23.12.32	„	9.1.33	17	12·5	14	26·5	47·1–52·9
25.12.32	„	9.1.33	15	9	13	22	40·9–59·1
26.12.32	„	9.1.33	14	10·5	16	26·5	39·6-60·4
29.12.32	„	9.1.33	11	11	14	25	44 –56
30.12.32	„	9.1.33	10	11·5	14	25·5	45 –55
6.12.32	397	20.12.32	14	19·5	8	27·5	70·9–29·1
7.12.32	„	21.12.32	14	19	8·5	27·5	72·7–27·3
9.12.32	„	23.12.32	14	23·5	8·5	32	73·4–26·6
11.12.32	„	23.12.32	12	19	10·5	29·5	64·4–35·6
13.12.32	„	23.12.32	10	18	13	31	58 –42
14.12.32	„	23.12.32	9	14	15	29	48·2–51·8
15.12.32	„	23.12.32	8	17	14·5	31·5	53·9–46·1
16.12.32	„	23.12.32	7	16·5	15	31·5	52·6–47·4
18.12.32	„	23.12.32	5	16	15	31	51·6–48·4
19.12.32	„	23·12·32	4	14	17	31	45·1-54·9
21.12.32	„	23·12·32	2	16·5	16·5	33	50—50
10.12.32	„	23.12.32	13	19·5	9·5	29	67·2–32·8

Date.	No.	Date of Test.	Interval.	Thin Albumin.	Thick Albumin.	Total Albumin.	Percentage of Thin to Thick Albumin.
			Days.	c.cs.	c.cs.	c.cs.	% %
22.12.32	397	9.1.33	18	23·5	5	28·5	82·4–17·6
23.12.32	„	9.1.33	17	27·5	4	31·5	87·3–12·7
25.12.32	„	9.1.33	15	17·5	11	28·5	61·4–38·6
29.12.32	„	9.1.33	11	22·5	9	31·5	71·4–28·6
30.12.32	„	9.1.33	10	20	10	30	66·6–33·4
1.1.33	„	9.1.33	8	17·5	12·5	30	58·3–41·7
7.12.32	354	21.12.32	14	10	17·5	27·5	36·3–63·7
8.12.32	„	22.12.32	14	9·5	15	24·5	38·7–61·3
10.12.32	„	23.12.32	13	9·5	17·5	27	35·1–64·9
12.12.32	„	29.12.32	17	10	17·5	27·5	36·3–63·7
15.12.32	„	5.1.33	21	12·5	12·5	25	50 –50
17.12.32	„	5.1.33	19	9·5	12·5	22	43·1–56·9
18.12.32	„	5.1.33	18	10	16	26	38·4–61·6
19.12.32	„	10.1.33	21	13·5	9·5	23	58·7–41·3
21.12.32	„	10.1.33	19	12·5	13·5	26	48 –52
22.12.32	„	10.1.33	18	11	13	24	45·8–54·2
25.12.32	„	10.1.33	15	10·5	15·5	26	40·3–59·7
26.12.32	„	10.1.33	14	9·5	16	25·5	37·2–62·8
27.12.32	„	10.1.33	13	11·5	16	27·5	41·8–58·2
28.12.32	„	10.1.33	12	9·5	12·5	22	43·1–56·9
30.12.32	„	10.1.33	10	9·5	16	25·5	37·2–62·8
1.1.33	„	10.1.33	9	10	18	28	35·7–64·3
6.12.32	342	20.12.32	14	9·5	15	24·5	38·7–61·3
7.12.32	„	21.12.32	14	10·5	13·5	24	43·7–56·3
9.12.32	„	22.12.32	13	11	14·5	25·5	43·1–56·9
13.12.32	„	5.1.33	23	11·5	12·5	24	47·9–52·1
14.12.32	„	5.1.33	22	9	13	22	40·9–59·1
20.12.32	„	11.1.33	22	13·5	10	23·5	57·4–42·6
23.12.32	„	11.1.33	19	10	14	24	41·6–58·4
25.12.32	„	11.1.33	17	13	11·5	24·5	53 –47
16.12,32	„	11.1.33	26	9	13·5	22·5	40 –60
19.12.32	„	11.1.33	25	12·5	10·5	23	54·3–45·7
26.12.32	„	11.1.33	16	11	14·5	25·5	43·1–56·9
29.12.32	„	1.11.33	13	12	12·5	24·5	48·9–51·1
30.12.32	„	11.1.33	12	11	13	24	45·8–54·2
7.12.32	362	21.12.32	14	15	9	24	62·5–37·5
8.12.32	„	22.12.32	14	12	9·5	21·5	55·8–44·2
10.12.32	„	22.12.32	12	16·5	7·5	24	68·7–31·3
11.12.32	„	24·12·32	13	13·5	9·5	23	58·7–41·3
13.12.32	„	3.1.33	21	17	8	25	68 –32
14.12.32	„	9.1.33	26	18	5	23	78·2–21·8
16.12.32	„	9.1.33	24	15	6·5	21·5	69·7–30·3
17.12.32	„	9.1.33	23	15·5	6	21·5	72 –28
19.12.32	„	9.1.33	21	16·5	5·5	22	75 –25
21.12.32	„	9.1.33	19	18·5	6·5	25	74 –26
22.12.32	„	9.1.33	18	14	8·5	22·5	62·2–37·8
23.12.32	„	9.1.33	17	15	7·5	22·5	66·6–33·4

Date.	No.	Date of Test.	Interval.	Thin Albumin.	Thick Albumin.	Total Albumin.	Percentage of Thin to Thick Albumin.
			Days.	c.cs.	c.cs.	c.cs.	% %
25.12.32	362	9.1.33	15	14	9	23	60·8–39·2
27.12.32	,,	9.1.33	13	13	9·5	22·5	57·7–42·3
28.12.32	,,	9.1.33	12	13·5	9·5	23	58·7–41·3
30.12.32	,,	9.1.33	10	12	11	23	52·1–47·9
6.12.32	399	20.12.32	14	14	12	26	53·8–46·2
8.12.32	,,	22.12.32	14	15·5	9	24·5	63·2–36·8
9.12.32	,,	22.12.32	14	14	10·5	24·5	57·1–42·9
12.12.32	,,	29.12.32	17	17·5	7·5	25	70–30
13.12.32	,,	3.1.33	21	21	4	25	84 –16
15.12.32	,,	3.1.33	19	15·5	9	24·5	63·2–36·8
17.12.32	,,	3.1.33	17	17	8	25	68 –32
18.12.32	,,	3.1.33	16	18	9	27	66·6–33·4
19.12.32	,,	3.1.33	15	17	9	26	65·3–34·7
21.12.32	,,	3.1.33	13	14	11	25	56 –44
22.12.32	,,	3.1.33	12	12	13·5	25·5	47 –53
25.12.32	,,	3.1.33	9	12·5	11	23·5	53·1–46·9
26.12.32	,,	3.1.33	8	12·5	13	25·5	49 –51
28.12.32	,,	3.1.33	6	11·5	14·5	26	44·2–55·8
27.12.32	,,	10.1.33	17	16	7·5	23·5	68 –32
30.12.32	,,	10.1.33	14	12	14	26	46·1–53·9
1.1.33	,,	10.1.33	9	11·5	12·5	24	47·9–52·1

APPENDIX 3.

CANDLED "WATERY WHITE" EGGS, *Ex* DURBAN.

Date.	No.	Weight of Egg.	Weight of Shell.	Percentage of Shell to Egg Weight.	Thin Albumin.	Thick Albumin.	Total Albumin.	Percentage Thin to Thick Albumin.
		gms.	gms.	%	c.cs.	c.cs.	c.cs.	% %
20.10.32	1	59·671	6·5	10·8	9	15·5	24·5	36·7–63·3
„	2	58·036	5·7	9·9	10·5	13	23·5	44·2–55·8
„	3	57·016	5·2	9·2	12	11	23·3	51·5–48·5
„	4	59·109	6·1	10·4	13	12	25	52 –48
„	5	62·709	5·7	9·1	12	18	30	40 –60
„	6	53·891	5·1	9·5	12	11	23	52·1–47·9
„	7	54·362	5·6	10·3	10	15	25	40 –60
„	8	56·623	5·4	9·5	7	15	22	31·8–68·2
„	9	56·730	5·6	9·9	8	17	25	32 -68
„	10	64·429	6·9	10·7	15	12	27	54·8–45·2
„	11	58·399	5·7	9·8	11	15	26	42·3–57·7
„	12	56·951	5·5	9·8	12	12·5	24·5	48·9–51·1
„	13	60·540	6·3	10·4	16	10	26	61·5–38·5
„	14	68·197	6·7	9·8	16	13	29	55·1–44·9
„	15	52·419	5·9	11·1	11	12	23	47·9–52·1
„	16	50·296	5·5	10·9	10	11	21	47·6–52·4
29.10.32	17	60·641	6·535	10·7	12	15	27	44·4–55·6
„	18	53·041	5·615	10·5	16	7	23	69·5–30·5
„	19	57·036	6·585	11·5	8·5	17·5	26	32·6–67·4
„	20	58·826	5·248	8·9	13·5	14·5	28	48·2–51·8
„	21	66·351	6·555	9·8	16	18	34	47 53
„	22	58·651	5·495	9·3	11	13·5	24·5	44·9–55·1
„	23	62·751	6·338	10	16	11	27	59·2–40·8
„	24	61·671	7·090	11	13	17	30	43·3–56·7
„	25	51·746	5·348	10·3	9·5	13	22·5	42·2–57·8
„	26	58·461	5·665	9·6	11·5	17·5	29	39·6–60·4
„	27	51·941	5·561	10·7	15	13	28	53·5–46·5
„	28	49·696	4·745	9·5	10·5	12·5	23	41·2–58·8
„	29	54·406	5·233	9·6	11	17	28	39·2–60·8
„	30	50·126	5·338	10·6	10	12	22	45·4–54·6
„	31	49·501	5·655	11·4	6·5	14·5	21	30·9–69·1
9.11.32	32	54·811	5·870	10·7	9	14·5	23·5	38·2–61·8
„	33	61·226	6·755	11	9·5	17	26·5	35·8–64·2
„	34	54·501	5·785	10·6	13·5	9·5	23	58·7–41·3
„	35	50·956	4·955	9·7	13·5	10	23·5	57·4–42·6
„	36	46·651	4·605	9·8	8·5	12	20·5	41·4–58·6
„	37	54·481	5·055	9·2	9	16·5	25·5	35·2–64·8
„	38	58·176	6·770	11·6	11·5	14	25·5	45 –55
„	39	59·906	5·945	9·9	10	17·5	27·5	36·3–63·7
„	40	54·316	4·910	9	6·5	19	25·5	25·4–74·6
„	41	67·701	6·273	9·2	14	18·5	32·5	43 – 57
„	42	54·901	5·751	10·4	14·5	8·5	23	63 –37
„	43	56·656	6·007	10·6	12	13	25	48 –52
„	44	60·106	6·578	10·9	11	17·5	28·5	38·5–61·5
„	45	55·806	5·390	9·6	15·5	9·5	25	62 -38
„	46	64·111	6·672	10·4	16·5	13·5	30	55 -45
„	47	58·541	5·672	9·6	14	14·5	28·5	49·1–50·1
„	48	54·656	6·450	11·8	8·5	12·5	21	40·4–59·6
„	49	54·201	5·762	10·6	9·5	16	25·5	37·2–62·8
14.11.32	50	63·021	7·155	11·3	12	16	28	42·8–57·2
„	51	56·791	5·358	9·4	10	15	25	40 –60
„	52	55·956	5·470	9·7	18	7	25	72 –28
„	53	59·526	6·428	10·7	12	16·5	28·5	42·1–57·9
„	54	50·499	5·770	11·4	13	10·5	23·5	55·3–44·7
„	55	50·281	4·675	9·3	8	14·5	22·5	35·5–64·5
„	56	59·476	6·200	10·4	16·5	11	27·5	60– 40

Date.	No.	Weight of Egg.	Weight of Shell.	Percentage of Shell to Egg Weight.	Thin Albumin.	Thick Albumin.	Total Albumin	Percentage Thin to Thick Albumin.
		gms.	gms.	%	c.cs.	c.cs.	c.cs.	% %
„	57	54·381	5·095	9·3	14·5	13·5	28	51·7–48·3
„	58	65·046	5·735	8·8	15·5	18·5	34	45·5–54·5
„	59	60·593	7·285	12	10·5	14·5	25	42 –58
„	60	52·161	5·263	10	10·5	12	22·5	46·6–53·4
„	61	55·503	6·776	12·1	8	14·5	22·5	35·5–64·5
„	62	55·221	5·095	9·2	7·5	14·5	22	34 –66
„	63	59·631	5·977	10	10	16	28	35·7–64·3
„	64	56·341	6·420	11·4	10	15·5	25·5	39·2–60·8
14.11.32	65	51·846	4·220	8·1	8	15·5	23·5	34 –66
„	66	58·101	6·785	11·6	13	13·5	26·5	49 –51
„	67	55·601	5·170	9·3	9·5	16·5	26	36·5–63·5
„	68	61·951	6·140	9·9	16·5	12	28·5	57·8–42·2
„	69	56·621	5·870	10·3	11·5	15·5	27	42·5–57·5
„	70	56·401	—	—	—	—	—	—
„	71	47·907	5·740	11·9	10	9	19	52·6–47·4
„	72	52·761	5·980	11·3	10	13·5	23·5	42·5–57·5
„	73	56·955	—	—	15	11 5	26·5	56·6 43·4
„	74	52·66	—	—	13	10	23	56·5–43 5
„	75	59·35	—	—	18	7	25	72 –28
„	76	66·325	—	—	20·5	11	31·5	65 –35
„	77	59·05	—	—	—	—	—	—
„	78	54·47	—	—	6	19	25·5	23·5–76·5
„	79	61·155	—	—	18·5	9	27·5	67·2–32·8
„	80	58·455	—	—	13·5	19	32·5	41·5–58·5
„	81	62·15	—	—	14·5	17	31·5	46 –54
„	82	59·895	—	—	12	16	28	42·8–57·2
„	83	66·155	—	—	13	14	27	48·1–51·9
„	84	55·06	—	—	13	11·5	24·5	53 -47
„	85	56·865	—	—	11	17·5	28·5	38·5–61·5
„	86	57·675	—	—	11	17	28	39·2–60·8
„	87	54·425	—	—	9	14	23	39·1–60·9
„	88	53·34	—	—	15	9	24	62·5–37·5
„	89	58·625	—	—	9 5	14	23·5	40·4–59·6
„	90	53·55	—	—	4	18	22	18·1–81·9
„	91	52·015	—	—	12·5	12·5	25	50 –50
„	92	42·755	—	—	9	10	19	47·3–52·7
„	93	49·505	—	—	13·5	7·5	21	64·2–35·8
„	94	49·92	—	—	16·5	6·5	23	71·7-28·3
„	95	41·145	—	—	6	12	18	33·3–66·7
29.11.32	96	61·901	—	—	25	3	28	89·2–10·8
„	97	56·061	—	—	14	11	25	56 –44
„	98	61·931	—	—	14	15	29	48·2–51·8
„	99	65·601	—	—	17	14	31	54·9–45·1
„	100	61·971	—	—	17·5	11·5	29	60·3–39·7
„	101	56·271	—	—	11	14·5	25·5	43·1–56·9
„	102	59·666	—	—	13	14·5	27·5	47·2–52·8
„	103	66·271	—	—	—	—	—	—
„	104	59·781	—	—	11·5	15	26·5	43·3–56·7
„	105	66·386	—	—	12	24	36	33·3–66·7
„	106	56·411	—	—	9·5	14	23·5	40·4–59·6
„	107	58·671	—	—	9·5	20	29·5	32·2–67·8
„	108	63·161	—	—	12·5	18·5	31	40·3–59·7
„	109	58·061	—	—	11·5	14	25·5	45 –55
„	110	63·321	—	—	16·5	16·5	33	50 –50
„	111	56·821	—	—	17·5	9	26·5	66 –34
„	112	58·731	—	—	13	16	29	44·8–55·2

Date.	No.	Weight of Egg.	Weight of Shell.	Percentage of Shell to Egg Weight.	Thin Albumin.	Thick Albumin.	Total Albumin.	Percentage Thin to Thick Albumin.
		gms.	gms.	%	c.cs.	c.cs.	c.cs.	% %
„	113	54·941	—	—	15·5	10·5	26	59·6–40·4
„	114	57·911	—	—	15	10	25	60 –40
„	115	60·291	—	—	14	15	29	48·2–51·8
„	116	51·111	—	—	11	11·5	22·5	48·8–51·2
„	117	49 671	—	—	8	14	22	36·3–63·7
„	118	49·321	—	—	11·5	11	22·5	51·1–48·9
7.12.32	119	68·386	6·435	9·4	20	12	32	62·5–37·5
„	120	64·981	6·807	10·4	12	20	32	37·5–62·5
„	121	62·706	6·06	9·5	15·5	14·5	30	51·6–48·4
„	122	58·801	5·907	10	11·5	11	22·5	51·1–48·9
„	123	51 426	4·202	8·1	20	2 5	22·5	88·8–11·2
„	124	52·656	5·175	9·8	11	13·5	24·5	44·8–55·2
„	125	57·961	6·095	10·5	8·5	19·5	28	30·3–69·7
„	126	58·746	5·680	9·6	10	16·5	26·5	37·7–62 3
„	127	60·481	6·2	10·2	16·5	12·5	29	56·9–43·1
„	128	60 476	5·9	9·7	16·5	14·5	31	53·2 46·8
„	129	58 981	5·495	9·3	15 5	14	29·5	52·5–47·5
„	130	54·561	5·220	9 5	8·5	17	25·5	33·3–66·7
„	131	56 686	4·807	8·4	15·5	12	27·5	56·3–43 7
„	132	60·321	6·395	10·5	11·5	17·5	29	39·6–60 4
„	133	52 666	4·303	8·1	7 5	13·5	21	35 7–64·3
„	134	59·756	5·745	9·6	14·5	13	27·5	52·7–47 3
„	135	52·191	6·170	11·8	14·5	8·5	23	63 –37
„	136	53·716	5·610	10·4	15	11	26	57·6–42·4
„	137	50·116	4 675	9·3	7	12 5	19·5	35·8–64·2
„	138	48 036	5·270	10·9	9·5	11	20·5	46·3–53·7
„	139	45·926	3·250	7	7·5	15	22·5	33·3–66·7
„	140	68·018	—	—	14·5	19·5	33	43·9–56·1
„	141	64·250	—	—	8	16·5	24·5	32·6–67·4
„	142	54·790	—	—	19	3	22	86·3–13 7
„	143	61·598	—	—	27	3	30	90 –10
„	144	59·705		—	19·5	8	27·5	70·9–29·1
„	145	55·038	—	—	14·5	10	24·5	59·1–40·9
„	146	51·085	—	—	11	8	19	57·9–42·1
„	147	56·149	—	—	16	11	27	59·2–40·8
„	148	60 905	—	—	13·5	15	28·5	47·3–52·7
„	149	59·2	—	—	18	12	30	60 –40
„	150	58·075	—	—	9	16	25	36 –64
„	151	55·075	—	—	9·5	13	22·5	42·2–57·8
„	152	63·518	—	—	15·5	16	31·5	49·2–50·8
„	153	59·555	—	—	18	9	27·5	65·4–34·6
„	154	53·58	—	—	9	15	24	37·5–62 5
„	155	68·268	—	—	24·5	7·5	32	76·5–23·5
„	156	57·388	—	—	12·5	13·5	26	48 –52
„	157	57·701	—	—	11	13·5	24 5	44·8–55·2
„	158	52·189	—	—	9·5	14	23·5	40·4–59·6
„	159	47·74	—	—	5·5	11·5	17	32 3–67·7
28.12.32	160	—	—	—	10	15	25	40 –60
„	161	—	—	—	13	16	29	44·8–55·2
„	162	—	—	—	5	14	19	26·3–73·7
„	163	—	—	—	15	9	24	62·5–37·5
„	164	—	—	—	19	6·5	25·5	74·5–29·5
„	165	—	—	—	15·5	10	25·5	60·7–39·3
„	166	—	—	—	10·5	11·5	22	47·7–52·3
„	167	—	—	—	17·5	10	27·5	63·6–36·4
„	168	—	—	—	7	8	15	46·6–53·4

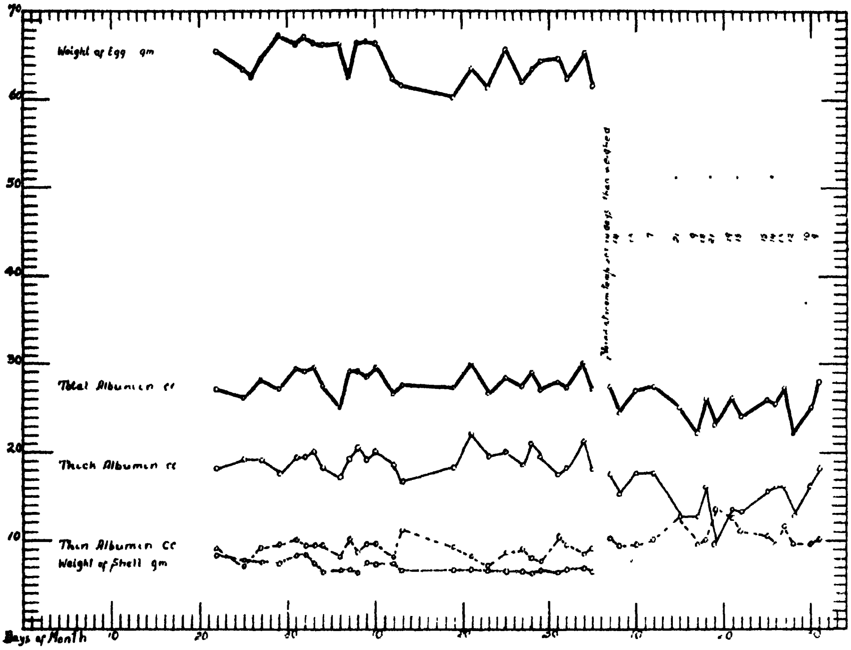
Date.	No.	Weight of Egg.	Weight of Shell.	Percentage of Shell to Egg Weight.	Thin Albumin.	Thick Albumin.	Total Albumin.	Percentage Thin to Thick Albumin.
		gms.	gms.	%	c.cs.	c.cs.	c.cs.	% %
,,	169	—	—	—	10·5	7·5	18	58·3–41·7
,,	170	—	—	—	18	8	26	69·2–30·8
,,	171	—	—	—	7·5	9·5	17	44·1–55·9
,,	172	—	—	—	9·5	11	20·5	46·3–53·7
,,	173	—	—	—	19·5	7·5	27	72·2–27·8
,,	174	—	—	—	8·5	19	27·5	30·9–69·1
,,	175	—	—	—	16·5	9	25·5	64·7–35·3
,,	176	—	—	—	11	8	19	57·9–42·1
,,	177	—	—	—	16	13	29	55·1–44·9
,,	178	—	—	—	7·5	14	21·5	34·8–65·2
,,	179	—	—	—	14·5	11	25·5	56·8–43·2
,,	180	—	—	—	23·5	4·5	28	83·9–16·1
,,	181	—	—	—	12·5	12	24·5	51 –49
11.1.33	182	—	—	—	12·5	13	25·5	49 –51
,,	183	—	—	—	8·5	15·5	24	35·4–64·6
,,	184	·	—	—	15	8	23	65·2–34·8
,,	185	—	—	—	23	2·5	25·5	90·1– 9·9
,,	186	—	—	—	13·5	14·5	28	48·2–51·8
,,	187	—	—	—	15·5	6·5	22	70·4–29·6
,,	188	—	—	—	14·5	13	27·5	52·7–47·3
,,	189	—	—	—	15·5	7·5	23	67·3–32·7
,,	190	—	—	—	15	8	23	65·2–34·8
,,	191	—	—	—	14	12	26	53·8–46·2
,,	192	—	—	—	13·5	8·5	22	61·3–38·7
11.1.33	193	—	—	—	12	9·5	21·5	55·8–44·2
18.1.33	194	—	—	—	19	14·5	33·5	56·3–43·7
,,	195	—	—	—	12	14	26	46·1–53·9
,,	196	—	—	—	17·5	8·5	26	67·3–32·7
,,	197	—	—	—	10	16·5	26·5	37·7–62·3
,,	198	—	—	—	12·5	13·5	26	48 –52
,,	199	—	—	—	14·5	14·5	29	50 –50
,,	200	—	—	—	14·5	8·5	23	63 –37
,,	201	—		—	14·5	7	21·5	67·3–32·7
,,	202	—	—	—	15	9	24	62·5–37·5
,,	203	—	—	—	14·5	12	26·5	54·7–45·3
,,	204	—	—	—	13	15	28	46·4–53·6
,,	205	—	—	—	14	12·5	26·5	52·8–47·2
,,	206	—	—	—	8	12·5	20·5	39 –61
,,	207	—	—	—	11·5	14	25·5	45 –55
,,	208	—	—	—	12·5	5	17·5	71·4–28·6
,,	209	—	—	—	15	9·5	24·5	61·2–38·8
,,	210	—	—	—	15·5	12	27·5	56·3–43·7
,,	211	—	—	—	19·5	2	21·5	90·7– 9·3
,,	212	—	—	—	22·5	1·5	24	93·7– 6·3
,,	213	—	—	—	17·5	2	19·5	89·7–10·3
,,	214	—	—	—	12·5	10·5	23	54·3–45·7
,,	215	—	—	—	12·5	15	27·5	45·4–54·6
28.1.33	216	—	—	—	11	15·5	26·5	41·5–58·5
,,	217	—	—	—	8	21·5	29·5	27·1–72·9
,,	218	—	—	—	17	16	33	51·4–48·6
,,	219	—	—	—	9·5	15·5	25	38 –62
,,	220	—	—	—	14·5	11	25·5	56·8–43·2
,,	221	—	—	—	14·5	15	29·5	49·1–50·9
,,	222	—	—	—	13	12·5	25·5	50·9–49·1
,,	223	—	—	—	13	17	30	43·3–56·7
,,	224	—	—	—	15	19	34	44·1–55·9
,,	225	—	—	—	11	9·5	20·5	56·6–43·4

TABLE 1.

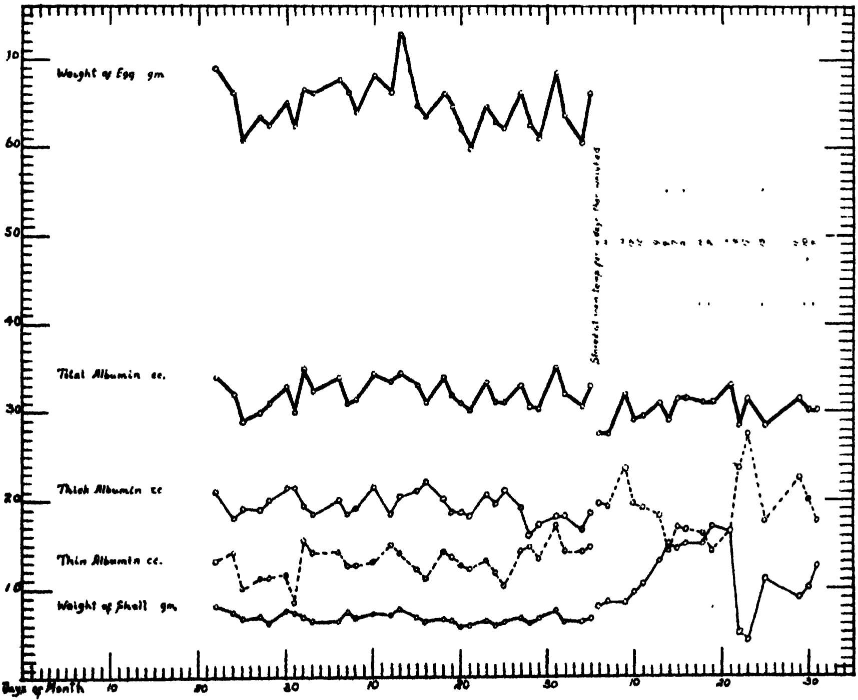
No. of Egg.	Date. Laid.	Temperature of Incubator.	Date of Testing.	No. of Days in Incubator.	Size of Air Sac at Testing. inches.	Description of Shake.	Description of Shell.	Description of Yolk after Test.	Description of Albumin, after Test.	pH on Testing.
9	22.4.32	37° C.	3.5.32	11	$1\frac{3}{8}$	Moderate..	Good quality	Flattened...	Apparently normal; watery white	8
10	„	„	„	11	$1\frac{5}{16}$	„	„	„ ..	Slightly thick, clear; watery white	7·8
11	23.4.32	37° C.	3.5.32	10	$1\frac{1}{16}$	Moderate..	Good quality	Flattened...	Slightly thick, clear; watery white	7·8
12	„	„	„	10	$1\frac{3}{8}$	Slight.....	Not too thick	„	Apparently normal; watery white	7·8
3	19.4.32	37° C.	27.4.32	8	$1\frac{5}{16}$	Slight.....	Good quality	Flattened...	Slightly turbid: watery white....	8
4	„	„	„	8	$1\frac{1}{8}$	„	„	„	Apparently normal: watery white	8
5	20.4.32	37° C.	28.4.32	8	$1\frac{3}{16}$	Moderate..	Good quality	Small round	Watery and clear: watery white	8
6	„	„	„	8	$1\frac{1}{4}$	Slight.....	„	„	„ „ „	8
7	21.4.32	37° C.	29.4.32	8	$1\frac{5}{16}$	Strong....	Thick, good	Flattened...	Watery and clear; watery white	8
8	„	„	„	8	$1\frac{1}{16}$	Moderate..	Good quality	„ ..	Watery and slightly turbid; watery white	8
13	25.4.32	„	3.5.32	8	$1\frac{1}{8}$	Slight.....	Not too thick	Rounded...	Apparently normal; watery white	8
14	„	„	„	8	$1\frac{1}{4}$	„	„	„ ...	„ „ „	8
1	18.4.32	37° C.	25.4.32	7	$1\frac{3}{16}$	Slight.....	Not too thick	Flattened...	Slightly turbid and watery white	7·5
2	„	„	„	7	$1\frac{1}{4}$	„	„	Rounded...	Apparently normal; watery white	7·5
28	10.5.32	37° C.	16.5.32	6	$1\frac{1}{4}$	Slight.....	Not too thick	Flattened...	Apparently normal; watery white	8
29	„	„	„	6	$1\frac{1}{16}$	„	„	„	Watery and clear; watery white	8
26	9.5.32	37° C.	14.5.32	5	$1\frac{1}{8}$	Slight.....	Not too thick	Flattened...	Slightly turbid; watery white	7·8
27	„	„	„	5	$1\frac{3}{16}$	„	Good quality	„ ..	Apparently normal; watery white	8

No. of Egg.	Date. Laid.	Temperature of Incubator.	Date of Testing.	No. of Days in Incubator.	Size of Air Sac at Testing. inches.	Description of Shake.	Description of Shell.	Description of Yolk after Test.	Description of Albumin, after Test.	pH on Testing.
30 31 31	11.5.32 „ „	37° C. „ „	16.5.32 „ 17.5.32	5 5 6	1 $\frac{11}{16}$ $\frac{15}{16}$	Slight..... Vigorous... „ ..	Not too thick Thick shell.. „ ..	Flattened... — —	Thin and clear; watery white — Broken, because unable to set up Watery white. Albumin membrane only detached	7·9 — 7·2
32 33	12.5.32 „	37° C. „	16.5.32 „	4 4	1 1	Slight..... „	Not too thick „	Rounded... „ ...	Clear and thin; watery white „ „ „	8 8
21 22	5.5.32 „	37° C. „	9.5.32 „	4 4	$\frac{7}{8}$ $\frac{7}{8}$	Slight..... Vigorous...	Fair......... Thick.......	Flattened... „ ..	Thick and clear; watery white Clear and thin; watery white	8 7·8
25	7.5.32	37° C.	11.5.32	4	$\frac{7}{8}$	Slight.....	Not too thick	Flattened...	Thick and clear; watery white	7·8
34 35	13.5.32 „	37° C. „	16.5.32 „	3 3	$\frac{15}{16}$ $\frac{15}{16}$	Slight..... Moderate..	Not too thick Fairly thick	Flattened... Flattened...	Fairly thick; watery white...... „ „	8 8
23 24	6.5.32 „	37° C. „	9.5.32 „	3 3	$\frac{11}{16}$ $\frac{3}{4}$	Slight..... „	Not too thick „	Small, round „	Thin and clear; watery white „ „ „	8 8
20 19	4.5.32 „	37° C. „	6.5.32 „	2 2	$\frac{5}{8}$ $\frac{3}{4}$	Slight..... Moderate..	Not too thick Fairly thick	Small, round „	Apparently normal; watery white „ „ „	8 8
15 16	2.5.32 „	37° C. „	3.5.32 „	1 1	$\frac{1}{2}$ $\frac{1}{2}$	Vigorous... „ ..	Fair......... „	Small, round „	Apparently normal; watery white „ „ „	7·8 8
17 17 18 18	3.5.32 „ 3.5.32 „	37° C. „ „ „	4.5.32 5.5.32 4.5.32 5.5.32	1 2 1 2	$\frac{1}{2}$ $\frac{5}{8}$ $\frac{1}{2}$ $\frac{5}{8}$	Vigorous... Slight..... Vigorous... Slight.....	Fair......... „ .... „ .... „	— Small, round — Small, round	*No watery white.* Apparently normal; watery white *No watery white.* Apparently normal; watery white	 7·8 7·8
36 37	21.5.32 „	53° C. „	21.5.32 „	6 hrs. 1½ hrs.	— —	*Coo* *Partly*	*ked.* *Cooked.*	— —	— —	— —

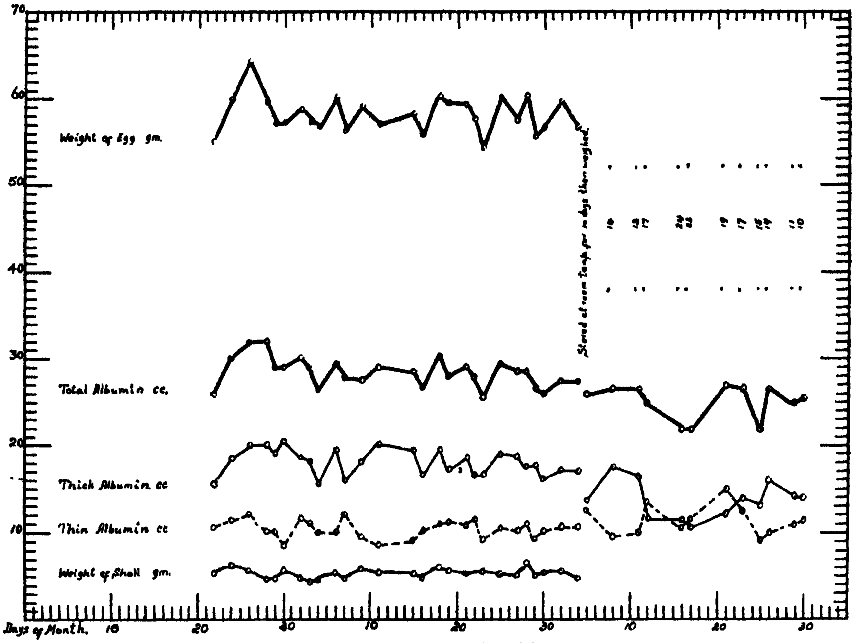
Watery white in last but one column refers to appearance over candle, albumin not measured.



Hen 354, Graph I.



Hen 397, Graph II.



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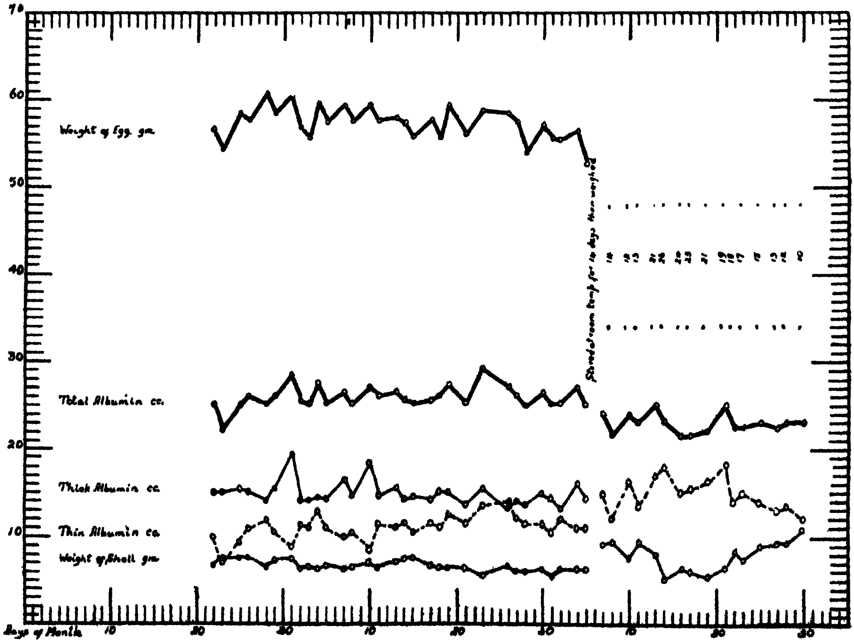

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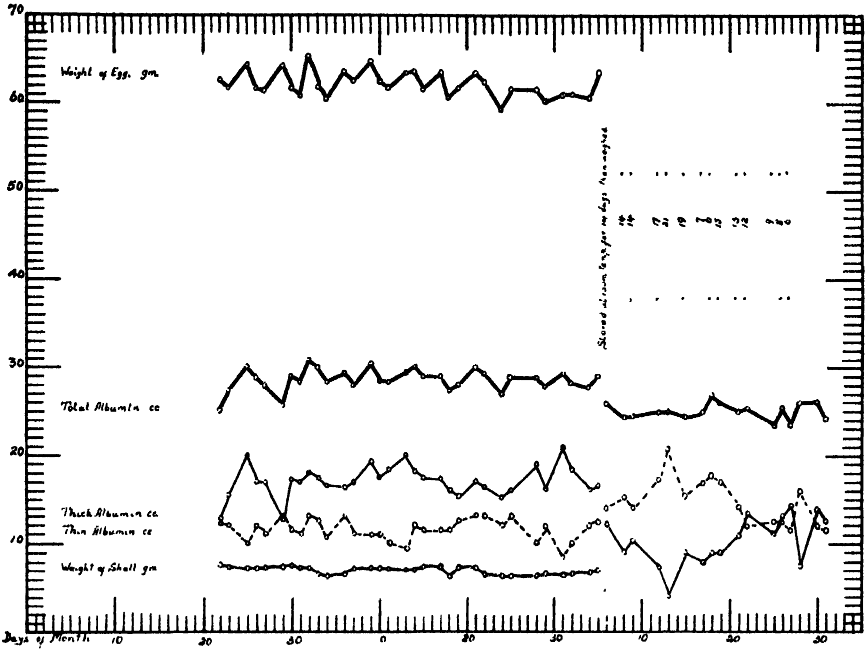

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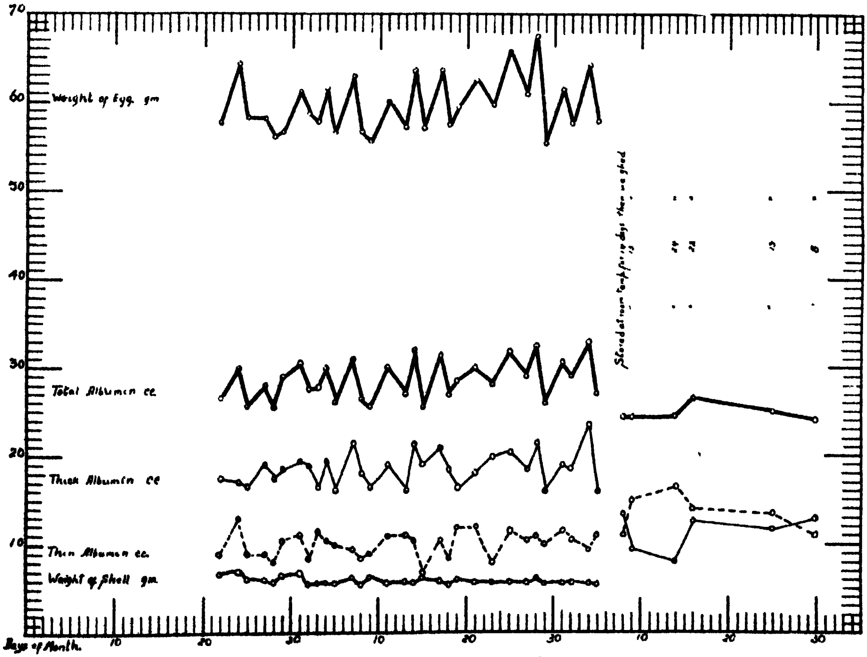

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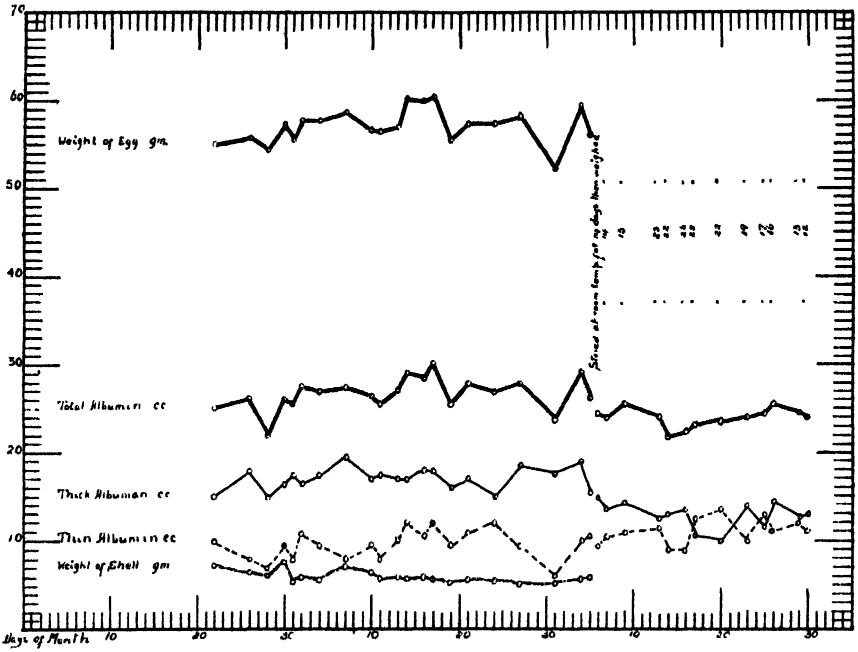
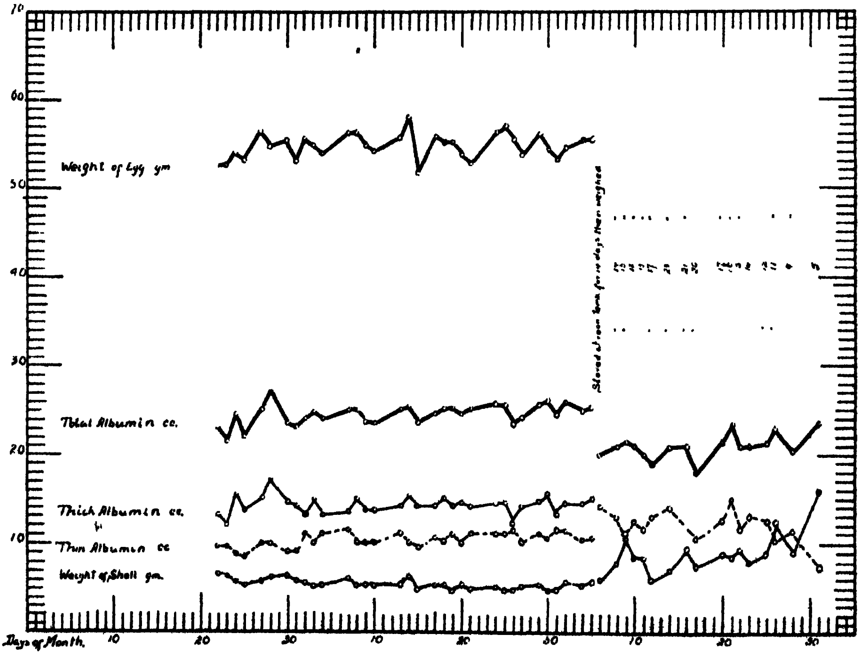


Hen 342, Graph VII.



Hen 947, Graph VIII.

Onderstepoort Journal of Veterinary Science and Animal Industry, Volume I, Number 2, 1933.

Influence of Regular Dipping on the Merino Sheep and its Fleece.

By Professor J. E. DUERDEN, M.Sc., Ph.D., Director of Wool Research;

G. S. MARÉ, B.Sc.(Agric.), Sheep and Wool Research Officer, Grootfontein; and

V. BOSMAN, M.Sc., Sheep and Wool Research Officer, Grootfontein.

INTRODUCTION.

A Merino sheep dipping experiment has been concluded at the Bathurst Experiment Farm. The latter is a sub-station of the Grootfontein School of Agriculture and is maintained for the purpose of investigating the many peculiar problems associated with the coastal areas as distinct from the Karroo. The Experiment Station is five miles inland at an altitude of 1,100 feet above sea-level. The rainfall is from 25-30 inches per annum and is distributed fairly evenly over the twelve months. The soil is sandy and the pasture is typical grass veld, Digitaria and Themeda species predominating.

Scattered bushes occur on the farm and part of it is overgrown with dense bush.

It is situated in a tick-infested region, where Merino sheep are farmed with difficulty on account of the prevalence of heartwater, the infection being carried by the bont tick (*Amblyomma hebraeum*).

The Bathurst district, like the rest of the coastal areas, is eminently suited for wool production and is known to grow wool of high quality which is much favoured by the trade. It has been asserted by older inhabitants that large flocks of Merino sheep flourished in the district before the advent of the tick and heartwater.

In their report " A Survey of Sheep Farming Conditions in the South-East Coastal Districts of the Cape," Warren, Maré and Roux (1928) mention that " The carrying capacity in the Bathurst district is about three sheep per morgen, though improved farms are said to carry five," and that " approximately 93 per cent. of losses among

European-owned sheep are due to disease; heartwater losses are most serious while blue tongue accounts for a relatively small percentage." Also that " heartwater is the most important problem which the sheep industry of the coastal belt is up against," and that " the most successful methods of combating the disease are the control measures advocated by the Veterinary Department, Pretoria." In these, weekly or fortnightly dipping of stock is recommended for the ultimate eradication of the tick. It is therefore evident that successful stock farming can only be maintained in the heartwater areas following upon tick elimination.

Until recently it was not considered advisable to dip Merino sheep carrying more than three to four months' wool growth. In its report " The Effect of Various Dips on Wool," the Department of Agriculture (1926) have recommended " that the sooner the dipping can be carried out after shearing, the less effect the dip is likely to have upon the wool." This means that successful introduction of Merinos into tick-infested areas would probably entail a regular weekly or fortnightly dipping for twelve months, which procedure seemed contrary to practice.

In order to ascertain whether the Merino could withstand regular dipping as that required for tick control, the above experiment was initiated.

PROBLEMS TO BE INVESTIGATED.

It was customary for farmers to make use of scrub cattle and non-woolled sheep for cleaning their farms of ticks. This type of stock was dipped either weekly or fortnightly without any harmful effects on the animal and its coat such as was expected with woolled sheep. In the case of Merinos no experimental data was available to show how such regular dipping would influence the animal and its fleece.

The present experiment was planned to conform as nearly as possible with the conditions and methods of dipping that were in vogue in the Bathurst district. The dip mostly in use was arsenite of soda; the strength of the solution varying according to whether seven-day or fortnightly dipping is followed.

In this experiment the sheep were to be subjected to regular seven-day dipping; the object in view was to establish, whether the Merino sheep could withstand and adapt itself to dipping at regular intervals if introduced into tick-infested areas, where regular dipping of stock is essential, and what effect such dipping would have on the fleece.

PLAN OF EXPERIMENT.

Ninety Merino hamels were used. Half of these were of the plain-bodied, long loose wool type, while half were of the wrinkly, short, dense wool type. The former were selected at the Grootfontein School of Agriculture, while the latter came from Queenstown. The object of having two distinct types was to ascertain whether there was any differential response between the types.

The 45 plain-bodied animals were divided into three groups of 15 each; the first group was dipped weekly in a solution of arsenite of soda of strength 2 lb. per 100 gallons water; the second were immersed weekly in water only, and these served as dipping controls; while the third lot were not dipped and served as general controls. A similar division and treatment was adopted with the 45 wrinkly animals.

There were thus 30 sheep which received a weekly dipping in arsenite of soda; 30 that were dipped in water; and 30 were not dipped. First dipping was applied on the 22nd January, 1930, three weeks after shearing.

The sheep were run in one flock on the Bathurst Experiment Station for the whole of the period of treatment. They received no extra feed except a lick, which consisted of: salt, bone-meal, sulphur and tobacco. Monthly dosing for gastrointestinal parasites was practised.

Two dipping tanks were used: one for water and one for arsenite of soda solution. The latter was tested and brought to the required strength before each dipping. All dipped animals received similar treatment as regards handling, and the immersion of each animal was timed for two minutes. The usual dipping precautions were observed such as: rain, extreme heat, driving before and after immersion, etc. On account of rain the animals were dipped only 39 times during the twelve months. In Table 1 the monthly rainfall is given as well as the number of dippings the animals were subjected to.

TABLE 1.

Month.	Rainfall in Inches.	No. of Dippings.
January	1·72	2
February	2·46	4
March	5·05	2
April	1·04	4
May	0·90	5
June	2·56	2
July	0·85	5
August	2·44	2
September	2·04	3
October	5·71	3
November	0·81	3
December	2·48	3
January	2·10	1

The condition of each sheep was recorded by monthly weighings, after the usual starvation period of 14-16 hours. Monthly observations were made on the occurrence of ticks. Wool samples were clipped on shoulder regions every month for laboratory analysis. All sheep were shorn at the end of twelve months and fleece weights recorded.

The wools were submitted for examination to wool buyers at Port Elizabeth and then sent to the British Wool Industries Research Association at Torridon, Leeds, for analysis and report.

TABLE 2.—*Group Live Weight Average in Pounds.*

Group.	Treatment.	Initial Shorn Weight 1930.	First Three Months.	Second Three Months.	Third Three Months.	Fourth Three Months.	Sheep Weight with Fleece 1931.	Fleece Weight.	Final Shorn Weight.	Percentage Increase of Body Weight.
A1*	Dipped in Ars. of Soda...........	90·4	92·5	88·5	89·8	98·4	97·6	10·4	87·2	Not significant.
B1†	Dipped in Ars. of Soda...........	72·0	80·7	83·9	86·4	96·4	96·1	10·9	85·2	18·3
A2*	Dipped in Water.................	89·4	93·1	87·6	89·3	95·8	96·1	8·9	87·2	Not significant.
B2†	Dipped in Water.................	74·2	81·6	88·5	86·6	96·3	96·2	10·4	85·8	15·6
A3*	Not Dipped......................	91·5	94·7	89·2	91·5	93·7	95·8	9·3	86·5	Not significant.
B3†	Not Dipped......................	70·8	80·9	84·8	87·6	94·7	95·1	11·8	83·3	17·7
A1 + B1	Dipped in Ars. of Soda...........	81·2	86·6	86·2	98·1	94·1	96·9	10·6	86·3	Not significant.
A2 + B2	Dipped in Water.................	81·8	87·4	85·6	87·9	96·1	96·2	9·7	86·5	„ „
A3 + B3	Not Dipped......................	81·2	87·9	86·5	89·6	95·9	95·5	10·6	84·9	„ „

* A1, A2, and A3 plain bodied, long loose woolled type

† B1, B2 and B3 wrinkly bodied short dense woolled type.

EXPERIMENTAL RECORDS AND RESULTS.

A.—CONDITION OF THE SHEEP.

Sheep weights varied somewhat during the course of the year, according to the feeding value of the veld. The 45 plain body sheep were in good condition at the commencement of the experiment, and the shorn weight in all groups at the end was not significantly different from that at the beginning. The wrinkly sheep on the other hand were in low condition at the beginning, and after a year showed an increase of fifteen to eighteen per cent. over the initial weights. At the conclusion, the three differentially treated groups, i.e. the arsenite dipped, water dipped and not dipped showed no significant difference over the initial weights.

In Table 2 are given the average three-monthly weights of the groups throughout the year; also the weight of the fleece in the greasy state, and the shorn weight of the group at the end of the period of treatment. Comparisons of the latter are made in the last column of the table, which establish that the sheep have not suffered adversely in condition after a year's weekly dipping when compared with the undipped animals.

B.—MORTALITY.

As regards mortality it is of significance that all the sheep dipped weekly in arsenite of soda survived. Of the thirty water-dipped controls, three died, while five deaths occurred among the thirty undipped controls, one of which was from heartwater. It must be observed that though the Bathurst Experiment Farm is situated in a tick-infested area, and that heartwater is prevalent, ticks have been largely eliminated from the farm, otherwise the mortality in the controls from heartwater would doubtless have been greater. This, however, in no way detracts from the value of the experiment, which was designed only to ascertain if Merino sheep could withstand weekly dipping while producing a full year's wool growth. A few odd ticks were found on the undipped and water-dipped controls, but none occurred on the arsenite dipped sheep. The latter were also free from blowfly trouble, whereas the controls were frequently struck.

Summarising, we may say that the experiment has proved that, under conditions similar to those prevailing at Bathurst, Merino sheep can be dipped weekly in the seven-day solution of arsenite of soda over a period of twelve months without any harmful effects to the animals.

C.—FLEECE.

It has been shown above that the dipping had no marked effect on the condition of the sheep, but as regards the fleece, differences have been established in some of the characteristics.

(a) *Weights.*

In Table 2 are given the fleece weights as shown in the greasy state. There is no significant change between dipped and undipped fleeces. Small differences that do occur may be due to incidentals of the greasy weights.

(b) *Fibre Fineness.*

As regards fibre fineness and quality of the wools in the different groups, measurements show no significant difference among the groups. In Table 3 are given mean fibre thickness and quality number.

TABLE 3.

Fibre Thickness.

Lot.	Mean Thickness. 1929.	Quality Number.	Treatment.	Mean Thickness. 1930.	Quality Number.
A	19·39μ	66's	Dipped Ars. Soda...	19·90μ	66's
B	19·54μ	66's	Dipped Water.......	20·00μ	66's
C	20·45μ	64's	Not Dipped.........	20·49μ	64's

In the above table comparative wool measurements are given for two successive seasons. The 1929 growth was produced at Grootfontein and Queenstown respectively, and the 1930 shearing at Bathurst.

A slight thickening of fibres is observed in the Bathurst grown wool, but this is consistent for all three groups.

There has therefore been no significant change in fibre thickness due to dipping; in fact, the quality numbers of the wools remained constant.

(c) *Staple Length.*

Staple lengths of 1929 and 1930 shearings are given in Table 4. There is no significant difference in the groups due to experimental treatment.

TABLE 4.

Lot.	Mean Length. 1929.	Treatment.	Mean Length. 1930.
A	6·3	Dipped in Ars. Soda....................	6·7
B	6·3	Dipped in Water.......................	6·5
C	6·2	Not Dipped...........................	6·7

(d) *Coloration.*

As regards coloration the wool from the dipped sheep presents a very different appearance from that of the undipped. The stapling is partly lost and for the most part the wool is harsh, inelastic to the feel, and unattractive in appearance. The foreign matter, consisting of sand and dirt, also extends more or less throughout the length of the staple, instead of being restricted to near the tip. The distribution gives a dull, dark appearance to the whole fleece, very different from the light, bright character of the undipped wool. Little or no difference however can be discovered when comparing the wool from the arsenite dipped sheep with that of the water dipped controls. Hence it can be assumed that deterioration was due to the water of the dip and not to the arsenite of soda.

A closer examination of the wool indicates the nature of the changes which have taken place. Mixed with the yolk of all wools is the substance known as suint. This is the dried perspiration of the sheep and is secreted by the sweat glands, while the grease proper is the waxy substance secreted by the fat glands. The suint is soluble in water, while the grease is not. It is manifest that by dipping the sheep in water the suint has been dissolved, and this has also affected the general distribution of the yolk. The water has penetrated the whole thickness of the fleece and either washed away the suint and yolk or transferred the particles of dirt with it, as well as any sediment from the dip. On the drying-up of the water in the sun, any yolk and suint would be re-deposited among the fibres, and the dirt particles along with them, producing a general discoloration and dullness. The natural arrangement of the staples is also partly disturbed, and the crimping somewhat obscured.

The arrangement of the individual fibres of the staples has not been markedly altered; they appear more closely bound together than usual, the "springy" feel and "life" of the wool being lost.

(c) *Deterioration and Values.*

In addition to the observations made in the research laboratory on the fleeces, these were submitted to experienced Wool Buyers and Brokers at Port Elizabeth for examination. It was admitted by them that they were not familiar with dipped wools as those presented, and consequently were somewhat reticent in being definite on merits and demerits of the dipped lots; although they were not in a position to value the wools with assurance there seemed a general agreement on the following:—

(*a*) That the dipped wools will require heavier scouring.

(*b*) That tops from the arsenite dipped wool will be either yellowish in colour or dingy in appearance.

(*c*) That the matting will affect the tare.

(*d*) That the clean yield will be higher for the arsenite and water dipped wools, than for the corresponding natural grease wools.

(*e*) a slight harshness in both arsenite and water dipped wools was admitted.

(*f*) A musty smell in the arsenite dipped wool was apparent, whereas the water-dipped wool retained the typical "sheepy" smell.

The Report by the British Wool Industries Research Association is appended in full and gives comments on the wools from the manufacturer's point of view.

ADDENDUM.

A.—Report by the British Wool Industries Research Association, Torridon, Leeds.

In accordance with the instructions forwarded after packing, two bales of wool arrived, consisting of:—

Lot A.—Fleeces from sheep dipped weekly in a solution of sodium arsenite.

Lot B.—Fleeces from sheep dipped weekly in plain water.

Lot C.—Fleeces from sheep undipped (controls).

These wools were examined in the greasy state at the mills of the Preston Street Combing Co., Bradford, by members of the British Wool Federation.

The experts were of the opinion that Lots A and B had lost considerably in appearance and handle. The appearance of A was probably better than that of B. Both A and B were inclined to be tender near the bottom of the staple. C was regarded as a good class of wool.

The order of excellence of the above lots was given as C, A, B.

It was decided that no further opinion could be given until the wools had been scoured and combed, and Messrs. John Smith & Sons kindly undertook to put the wool through these processes, and rendered the following report on the 23rd November, 1932:—

> "With reference to the three samples of South African wool which have been treated by a special sheep dip, we have arranged to have a top and a sample of noil from each lot to be forwarded to you to-morrow by road.
>
> "The following tables show the combing results for each lot:—

Lot.		Weight.	Tear.	Yield.
		lb.		%
C	Untreated Wool.....	115	11·66	33
A	Arsenite Dip........	197	13·93 to 1	42·6
B	Water Dip..........	140	10·2 to 1	40

> "In the above results the yield given is that of top and noil only, no allowance having been made for waste, etc. On account of the small weights of wool put through, not too much attention should be paid to these results. Difference in tear might just as easily be due to variations in the actual fleeces rather than to the effects of the dipping.

"Examination of the tops, however, shows a very marked difference; while Lot C may be classed as a 70's super 10-12 months' Cape of average strength, Lot A appears slightly longer and stronger in fibre, but, at the same time, rather low in quality. The general appearance seems to indicate that being slightly stronger, the longer fibres have not been broken quite so much as in Lot C. The additional strength, however, may be due to lower quality rather than to the effects of dipping.

"Compared with Lot C, Lot B is shorter and weaker, but about the same in quality. So far as general appearance and character are concerned, Lot C is a top made from good wools and shows good breeding, while Lots A and B give the appearance of being made from 'wool with a past'.

"You will notice that both these lots are apparently stained as the colouring is not the usual slightly yellow tint, apparent in most Cape wools. In view of the big difference between the treated and the untreated tops, we do not personally consider the matter is worth pursuing further. At the same time, in order to satisfy you in the matter, we are perfectly prepared to carry the wool through into yarn.

"A further point to take into consideration is, in our opinion, that there is as much as 1d. per pound difference in value between the noil from Lot C and that from Lots A and B, while in the top we estimate the difference at 2d. per pound."

(Signed) B. A. SMITH,
Director."

I. *Laboratory Report on Fineness and Contour.*

The average results of measurements made by C. G. Winson on selected samples each of 200 fibres from the three lots of wool are as follows:—

Lot.	Mean Cross-sectional Area x 10-6 Sq. Cms.	Standard Error.	Coeff. of Variation.	Mean A/B.
A (Sodium Arsenite Dip).......	3·92	0·10	% 36·9	1·21
B (Plain Water Dip)...........	3·83	0·10	38·6	1·23
C (Controls)..................	3·85	0·11	41·3	1·19

The difference in fineness of the three lots of wool are without significance. The difference in contour (A-B) or shape of fibre cross-section are also insignificant. There is thus, apparently, no effect of the dipping on fibre thickness and contour.

II. *Strength Tests.*

A number of fibres were examined in the extensometer by Mr. Van Wyk, and the following results obtained:—

Lot.	Breaking Load (Arb. Units).	Standard Error.	Coeff. of Variation.	Extension at Break.	Standard Error.	Coeff. of Variation.
			%	%		%
A	3·065	± ·23	± 50·0	28·35	± 2·05	± 46·3
B	2·969	± ·18	± 39·6	25·90	± 1·73	± 42·7
C	3·261	± ·26	± 45·6	28·89	± 2·27	± 43·7

From the above it is obvious that the trade opinion is confirmed. Lot B was considerably weaker than Lot A, which in turn was weaker than the control Lot C. The extensions show the same fact, and since little difference in average fineness ensued it is obvious that the above differences afford a true contention.

Thus the effect of the dip has been to weaken the fibres consistently.

III. *Sulphur Content.*

The sulphur content of these wools was determined by J. Barritt, and the values expressed on the dry weights were as follows:—

"A"	3.48 per cent.
"B"	3.44 per cent.
"C"	3.47 per cent.

The values are substantially the same.

In order to have a final trade opinion, the British Wool Federation of Bradford appointed a small sub-committee to examine the tops made by the Preston Street Combing Co. Their report is as follows:—

> "British Wool Federation,
> 14 Piccadilly,
> Bradford, 17*th December*, 1931.
>
> *Cape Wool.*
>
> "With regard to the samples of top and noil submitted by Messrs. John Smith & Co., Ltd., I have to report as follows:—
>
> "Messrs. Broadhead, Ayrton & Harland have to-day examined the tops made by the Preston Street Combing Co.
>
> "They are of opinion that Lot C is a good average 70's quality, good 10-12 months' length, and good colour. Lot A somewhat longer than C and a good full quality lower, sound staple, but bad colour. Lot B not quite so fine as Lot C and rather shorter, weak staple, and the worst colour.
>
> "They are of opinion that the fleeces sent are hardly comparable for the purpose you desire, as they cannot think that the arsenite or water dipping could affect the quality or length of the wool unless the dipping had some effect on the health of the sheep, which, of course, they are unable to determine."
>
> (Signed) W. HARRISON."

Conclusions.

" The final conclusions which must be arrived at from the above indicate that the water dip has in some way or other vastly deteriorated the wool either by affecting the animal or in some other way. Possibly there has been some effect on the health of the animal since the sulphur content of the wool has not been changed. On the other hand, the arsenite dip again had a deleterious effect since the wool is graded as a good full quality lower and is a bad colour.

" There is no doubt that these differences are real and we would say therefore that the effect of the arsenite dip has been found to deteriorate the wool.

(Signed) S. G. BARKER,
Director of Research."

B.—Analysis of Bathurst Water and Dip, as Supplied by the Chemistry Department of the Grootfontein School of Agriculture, Middelburg, Cape.

Analysis of Bathurst Water.

Salts in Solution.	Percentage by Weight.	Remarks.
Calcium Carbonate..................	·0375	Temporary.
Magnesium Carbonate...............	·0105	Hardness.
Magnesium Sulphate................	·0090	
Sodium Sulphate.....................	·0045	Permanent.
Sodium Chloride......................	·0780	Hardness.
	·1395	

Degrees of total hardness ... ... 43.5 per cent.
Degrees of permanent hardness ... 6.0 per cent.
Degrees of temporary hardness ... 37.5 per cent.

Analysis of Dip.

On four different occasions the manager of Bathurst Experiment Station submitted samples of the dip as used in the experiment.

The correct strength of a seven-day dip solution is ·16 per cent. As_2O_3.

The four samples analysed were as follows:—

Sample.	Percentage As_2O_3.	Percentage Error.
1.............	·167	+ 4·4
2.............	·167	+ 4·4
3.............	·156	− 2·5
4.............	·184	+ 15·0

DISCUSSION.

Regular weekly dipping in arsenite of soda does not influence the condition or body weight of the Merino adversely. The animals actually increased to the extent of 5·9 per cent. in live weight. Also, no deaths occurred, whereas at least one animal in the control group died of heartwater. Since no ticks were found on the arsenite dipped sheep in comparison with the controls it is to be concluded that the solution effectively checked tick infestation. This is of practical importance, for farmers who desire to farm Merino sheep on their tick-infested properties, can introduce Merinos immediately instead of postponing until such a time as the farm has been cleaned of ticks by means of cattle or non-woolled sheep. For two or three seasons such farmers will produce wool clips which have deteriorated somewhat in intrinsic value, but once the ticks are under control regular dipping can largely be dispensed with and normal wool production becomes possible.

The fleece is not influenced as regards greasy weight. This would indicate that the dipping process was not instrumental in washing out and removing the impurities from the fleece, but rather served to distribute these along the entire length of the staple. This, no doubt, was the cause of the unattractive appearance.

As regards fibre thickness in the three groups, the South African measurements (Table 3) indicate a slightly coarser wool in Group C, the undipped controls, whereas the Torridan results show slightly coarser wool in Group A, the arsenite dipped animals. Since both sets of measurements show only minor differences of no significance it is evident that dipping had no effect on fibre thickness.

Similarly in fibre contour, sulphur content and staple length no significant differences could be detected.

As regards tensile strength, members of the British Wool Federation consider that both the water- and arsenite-dipped wools are inclined to be tender near the bottom of the staple. This view was confirmed by tests carried out by Mr. van Wyk in the extensometer. The control lot was sounder than the dipped groups. It is of importance to note that the water-dipped wool was weaker than the arsenite-dipped. There is, however, a factor which must not be lost sight of in discussing the tensile strength of these wools. It is well known that any set-back to the health of a sheep results either in a complete break or a weakening in the fibres. The animals were in perfect health throughout, but during the four months preceding the final shearing, blow flies were very troublesome, and a number of the water-dipped animals as well as controls were struck; in fact, one sheep in the water-dipped group partly shed its fleece. None of the arsenite-dipped animals were attacked. This is a probable explanation why the wool from the water-dipped animals was less sound than that from the arsenite-dipped group.

For handle and appearance, the members of the British Wool Federation placed the three lots in order of merit: not dipped, arsenite-dipped, water-dipped. Messrs. John Smith & Sons, remarking on the dipped tops, state: " So far as general appearance and character are concerned, Lot C is a Top made from good wools and shows good breeding, while Lots A and B gave the appearance of being made from ' wool with a past ' ". Also, " you will notice that both these Lots are apparently stained as the colouring is not the usual slightly yellow tint apparent in most Cape wools."

There is therefore no doubt that in respect of tensile strength and colour the dipped wools deteriorated to some extent. This deterioration is evident to nearly the same degree in both the arsenite-dipped animals and the water-dipped lot. It can thus be inferred that sodium arsenite as such did little, if any, damage to the wool fibre. The deterioration as mentioned is most likely due to the hardness of the Bathurst water. It is still an open question whether, if rain-water were used, which is free from salts causing hardness, the wools would have deteriorated to such an extent.

As regards the monetary value, it is pointed out by Messrs. John Smith & Sons, who scoured and combed the wools, that " there is as much as 1d. per pound difference in value between the Noil from Lot C, and that from Lots A and B, while in the top they estimate the difference at 2d. per pound." At the time this statement was made (23rd November, 1931) the top values at Bradford of average Cape of 10-12 months was quoted at 25½d. per lb. A difference of 2d. per pound meant about 8 per cent. reduction in value, which is equivalent to approximately 1d. per pound in the grease for a 50 per cent. yielding wool. This difference in monetary value is not great, yet the same authority when referring to colouring, states that " in view of the big difference between the treated and the untreated tops we do not personally consider the matter is worth pursuing further."

Nowhere was any difference found in differential response between the two types of sheep used, namely, plain bodied, long loose wool, and wrinkly bodied, short dense wool.

In conclusion we wish to express our indebtedness to Mr. R. Paine, Government Veterinary Officer at Grahamstown, for valuable suggestions; also to Mr. F. C. Smith, manager of the Bathurst Experiment Station, for being responsible for the management, dipping and weighing of the sheep.

SUMMARY AND CONCLUSIONS.

1. A Merino sheep dipping experiment is described at the Bathurst Experiment Sation in the coastal region, which for the greater part is a tick-infested area.

2. Ninety Merino hamels were used. Half of the plain-bodied, long loose wool type and half the wrinkly short dense woolled type. Each group was divided into three lots of 15. The first lot dipped weekly in arsenite of soda of strength 2 pounds per 100 gallons of water, the second dipped in water, the third not dipped. There were thus thirty animals for each of the treatments.

3. The aim of the experiment was to establish whether the Merino sheep could withstand and adapt itself to dipping at weekly intervals for twelve months and what effect such dipping will have on the fleece.

4. Results show that weekly dipping does not influence the condition of the sheep as reflected in body weight.

5. All sheep dipped in arsenite of soda, survived after a year's treatment, and were always free from ticks and blowfly trouble.

6. Arsenite of soda had no influence on fleece weights, fibre thickness, staple length and fibre contour.

7. As regards colour, handle and appearance the wools dipped in arsenite and in water have deteriorated to some extent. Deterioration is practically of the same degree in the two dipped groups and presumably due to the hardness of the Bathurst water and not to the arsenite of soda dip.

8. As regards monetary value, as given by Messrs. John Smith & Sons, there is as much as 1d. per pound difference between the Noil from the control and that from the dipped lots, and in the Top a difference of 2d. per pound. The latter quotation at the time of estimating (23rd November, 1931), meant a difference of approximately of 1d. per pound in the grease for a 50 per cent. yielding wool, or a reduction in value of about 8 per cent.

9. There was no difference in response between the wrinkly bodied short dense woolled sheep and the plain bodied long loose woolled animals.

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Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 1, Number 2, 1933.

A Study of Wool from Merino Stud Rams.

By V. BOSMAN, M.Sc., Sheep and Wool Research Officer, Grootfontein School of Agriculture, Middelburg, Cape Province, and

P. S. BOTHA, M.Sc., Research Assistant, Rhodes University College, Grahamstown.

INTRODUCTION.

The wool characteristics of Merino stud rams are of significance to the sheep and wool industry of the Union, since it is the progeny of these rams that influence the flock breeder's wool clips. It is, therefore, of importance to the woolman to have an intimate knowledge of the wool produced by stud rams, and a study concerning these wools has been made.

Leading breeders in the Union were approached for wool samples from their Merino stud sires, and the collection thus obtained is representative of stud rams in use in 1931. The material is of particular value in that the history of the studs, pedigrees and breeding performances of the sheep concerned have, in most cases, been supplied. Although this paper does not consider the wools from a genetic point of view, it is intended as a preliminary to a more extensive study of wools from a breeding aspect. The investigation seeks to establish facts of a fundamental nature and gives a wool analysis from the point of view of crimping, fibre thickness, and degrees of variability.

NATURE OF MATERIAL.

Wool samples from 123 stud rams were analysed. These comprise material from 40 leading stud breeders and include many wools from valuable animals with noteworthy show and sale ring records. The samples were mostly of twelve months' growth and in the form of small staples cut from the shoulder region, wool from skin folds being avoided as far as possible as it has been shown that folds influence wool characteristics (Bosman, 1933) (¹). Shoulder samples were taken as a basis for comparison, as this class of wool forms the largest portion of the fleece and serves as an indication of the bulk of the fleece. (Duerden and Bell, 1931.)

The samples were fairly uniformly grown, although some showed a variation in crimping and fibre thickness along the staple, presumably due to differential nutritive treatment (Probst, 1926; Bosman and Maré, 1933) (²). Some showed the defects often met with in the wool from rams used for service, and known to the practical man as " service breaks."

(¹) " Influence of Skin Folds on Merino Wool."—Bosman, V. Report in preparation.

(²) " Influence of Feed on Merino Wool."—Bosman, V. and Maré, G. S. Report in preparation.

WOOL ANALYSIS AND METHODS.

The wool characteristics of crimping, fibre thickness, and thickness variability form the basis of the study. Crimping was measured in one region of the sample near the skin end and expressed as number of crimps per unit length.

For the estimation of fibre thickness, the wool grease was removed and small clippings taken through the same region as for crimping. The wool fragments thus obtained were intermingled in ether, mounted in Euparal and 500 fibres measured on a Zeiss Hegener Micro-Camera, the unit of measurement being 2·5 microns. The frequency distribution of fibre thickness of each sample was thus obtained and the mean thickness, standard deviation and coefficient of variability calculated. The sampling for fibre thickness and crimping differs slightly from the usual method employed in analysing wools for commercial purposes, in that in the latter, a mean of the whole length of the staple is obtained (Duerden, 1929). In the present investigation it was deemed necessary to confine measurements of crimping, fibre thickness and fibre distribution to one region only in order to eliminate regional differences that may be due to influences of nutrition and service.

When the whole length of the staple (where regions vary in thickness) is considered, the fibre distribution is different from that obtained when only one of these regions is studied.

EXPERIMENTAL RESULTS.

A.—Results of Analysis.

Table 1.

Sample.	Thickness in μ.	Qual. No. on Thickness.	Crimps per Inch.	Qual. No. on Crimps.	Stand. Dev.	C. of V. Per-cent.
39/11	16·95	90's	20–21	90's	0·863	5·1
17/1	18·01	70's	14–15	66's	1·208	6·7
10/2	18·05	,,	12–13	64's	1·470	8·1
21/2	18·18	,,	14–15	66's	1·340	6·2
15/4	18·39	,,	14–15	,,	1·529	8·3
21/1	18·19	,,	14–15	,,	1·007	5·5
23/3	18·50	,,	10–11	60's	1·097	5·9
44/6	18·87	,,	18–19	80's	1·071	5·7
14/1	19·00	66's	12–13	64's	1·153	6·1
17/2	19·18	,,	12–13	,,	1·562	8·0
10/1	19·44	,,	16–17	70's	1·228	6·3
5/3	19·44	,,	14–15	66's	1·356	7·0
3/7	19·54	,,	12–13	64's	1·401	7·2
6/2	19·73	,,	16–17	70's	1·523	7·7
12/1	19·91	,,	16–17	,,	1·367	6·9
2/1	20·03	64's	14–15	66's	1·277	6·4
1/2	20·10	,,	14–15	,,	1·288	6·1
32/1	20·21	,,	12–13	64's	1·207	6·0
7/2	20·34	,,	16–17	70's	1·248	6·1
12/5	20·38	,,	14–15	66's	1·492	6·9
1/3	20·39	,,	14–15	,,	1·304	6·4
16/1	20·59	,,	14–15	,,	1·134	5·5
28/2	20·63	,,	14–15	,,	1·544	7·5

TABLE 1—(*contd.*)

Sample.	Thickness in μ.	Qual. No. on Thickness.	Crimps per Inch.	Qual. No. on Crimps.	Stand. Dev.	C. of V. Per-cent.
37/2	20·95	„	14–15	„	1·221	5·8
19/2	20·95	„	12–13	64's	1·504	7·2
30/1	20·96	„	16–17	70's	1·456	6·9
26/1	20·99	„	16–17	„	1·588	7·6
39/8	21·05	„	12–13	64's	1·435	6·8
23/1	21·09	„	12–13	„	1·175	5·6
32/2	21·09	„	14–15	66's	1·382	6·6
39/3	21·14	„	18–19	80's	1·204	5·7
5/4	21·26	„	14–15	66's	1·463	6·9
23/4	21·28	„	14–15	„	1·374	6·4
18/3	21·32	60's	10–11	60's	1·575	7·5
41/7	21·44	„	12–13	64's	1·486	6·9
44/7	21·48	„	16–17	70's	1·594	7·4
19/10	21·53	„	14–15	66's	1·121	5·2
9/1	21·54	„	12–13	64's	1·206	5·6
6/1	21·54	„	10–11	60's	1·694	7·9
39/6	21·55	„	14–15	66's	1·609	7·5
3/2	21·58	„	16–17	70's	1·397	6·5
41/1	21·65	„	12–13	64's	1·425	6·6
10/4	21·70	„	14–15	66's	1·231	5·7
39/1	21·72	„	14–15	„	1·686	7·8
2/4	21·75	„	10–11	60's	1·302	6·0
10/3	21·75	„	16–17	70's	1·466	6·7
2/3	21·83	„	10–11	60's	1·533	7·0
25/5	21·89	„	12–13	64's	1·342	6·1
19/3	21·90	„	14–15	66's	1·227	5·6
39/2	21·91	„	12–13	64's	1·631	7·4
23/2	22·00	„	8–9	58's	1·430	6·5
39/5	22·09	„	8–9	„	1·465	6·6
18/4	22·16	„	10–11	60's	1·656	7·5
19/1	22·22	„	14–15	66's	1·351	6·1
9/2	22·42	„	12–13	64's	1·439	6·4
5/2	22·72	„	14–15	66's	1·518	6·6
4/3	22·72	„	12–13	64's	1·985	8·7
39/7	22·73	„	14–15	66's	1·524	6·7
3/1	22·76	„	12–13	64's	1·727	7·6
24/2	22·80	„	12–13	„	1·612	7·1
6/3	22·88	„	12–13	„	1·329	5·8
39/4	22·89	„	10–11	60's	1·833	8·0
1/5	22·91	„	10–11	„	1·703	7·4
11/1	23·02	58's	14–15	66's	1·472	6·4
38/1	23·03	„	10–11	60's	1·396	6·1
34/1	23·08	„	12–13	64's	1·296	5·6
37/1	23·12	„	14–15	66's	1·236	5·3
25/2	23·16	„	14–15	„	1·703	7·4
35/1	23·18	„	12–13	64's	1·516	6·5
34/4	23·21	„	10–11	60's	1·602	6·9
12/4	23·27	„	12–13	64's	1·381	5·9
34/3	23·41	„	12–13	„	1·757	7·5
8/2	23·42	„	12–13	„	1·605	6·9
4/1	23·45	„	14–15	66's	1·611	6·9
24/6	23·47	„	14–15	„	1·492	6·4
12/3	23·50	„	12–13	64's	1·549	6·6
33/3	23·56	„	8–9	58's	1·690	7·2
15/2	23·57	„	14–15	66's	1·701	7·2
23/8	23·58	„	14–15	„	1·666	7·1
2/2	23·63	„	12–13	64's	1·542	6·5

TABLE 1—(*contd.*)

Sample.	Thickness in μ.	Qual No. on Thickness.	Crimps per Inch.	Qual. No. on Crimps.	Stand. Dev.	C. of V. Per-cent.
8/3	23·64	,,	8–9	58's	1·524	6·4
41/3	23·68	,,	12–13	64's	1·528	6·5
41/2	23·78	,,	12–13	64's	1·448	6·1
41/5	23·79	,,	12–13	,,	1·600	6·7
41/4	23·85	,,	8–9	58's	1·868	7·8
41/6	23·92	,,	8–9	,,	1·654	6·9
33/4	23·93	,,	10–11	60's	1·508	6·3
5/6	23·98	,,	12–13	64's	1·773	7·4
4/2	23·99	,,	10-11	60's	1·732	7·2
35/7	24·11	,,	10-11	,,	1·647	6·8
3/3	24·16	,,	12–13	64's	1·746	7·2
14/2	24·20	,,	12–13	,,	1·365	5·6
15/3	24·35	,,	14–15	66's	1·411	5·8
15/5	24·36	,,	14-15	,,	1·338	5·5
7/1	24·40	,,	14–15	,,	1·727	7·1
12/2	24·43	,,	12-13	64's	1·431	5·9
18/1	24·51	,,	12–13	,,	1·827	7·5
24/1	24·52	,,	12–13	,,	1·695	6·9
5/5	24·60	,,	14-15	66's	1·554	6·3
3/6	24·60	,,	12-13	64's	1·810	7·4
26/2	24·64	,,	14–15	66's	1·451	5·9
8/1	24·65	,,	12–13	64's	1·423	5·8
39/10	24·66	,,	12-13	,,	1·451	5·9
33/1	24·72	,,	12–13	,,	1·582	6·4
34/5	24·82	,,	10–11	60's	1·748	7·0
40/1	24·91	,,	12–13	64's	1·857	7·5
24/3	24·94	,,	8–9	58's	1·591	6·4
15/1	25·09	,,	14–15	66's	1·443	5·8
1/4	25·18	,,	8–9	58's	1·793	7·1
29/1	25·30	,,	14–15	66's	1·498	5·9
25/3	25·41	,,	12–13	64's	1·486	5·8
25/4	25·43	,,	12–13	,,	1·528	6·0
34/2	25·65	56's	12–13	,,	1·563	6·1
20/2	25·65	,,	8–9	58's	1·998	7·8
44/8	25·65	,,	6–7	56's	2·064	8·0
39/9	25·68	,,	16–17	70's	1·629	6·3
20/1	25·81	,,	12–13	64's	1·942	7·5
25/1	25·87	,,	12–13	,,	1·742	6·7
28/1	26·26	,,	10–11	60's	1·683	6·4
1/1	27·06	,,	14–15	66's	1·667	6·2
33/2	27·33	,,	8–9	58's	1·566	5·7
18/2	27·58	,,	12–13	64's	1·988	7·2
33/5	27·62	,,	10–11	60's	2·094	7·6

B.—FIBRE THICKNESS AND CRIMPING.

In Table 1 is given the fibre thickness, quality number, standard deviation, and coefficient of variability, as well as the crimps and quality number on crimps of the samples. They are arranged in order of increasing fibre thickness, and present a range of from 16·96μ to 27·62μ, i.e. from 90's to 56's, which includes practically all the Merino qualities, as well as a few coarser ones. A similar result is obtained when quality number on crimps is compared. The range is from 20-21 per inch (a 90's) to 6-7 (a 56's). The proportions in which the qualities occur both on fibre thickness and on crimping in Table 1 are summarised in Table 2.

TABLE 2.

DISTRIBUTION OF QUALITY NUMBER ON THICKNESS.				DISTRIBUTION OF QUALITY NUMBER ON CRIMPING.			
Thickness Range.	Quality Number.	Fre-quency.	Per-cent.	Crimp Range.	Quality Number.	Fre-quency.	Per-cent.
16·2–17·0	90's	1	0·8	20–21	90's	1	0·8
17·0–17·9	80's	0	0	18–19	80's	2	1·6
17·9–18·9	70's	7	5·7	16–17	70's	10	8·1
18·9–20·0	66's	7	5·7	14–15	66's	38	30·9
20·0–21·3	64's	18	14·6	12–13	64's	45	36·6
21·3–23·0	60's	30	24·4	10–11	60's	16	13·0
23·0–25·5	58's	49	39·8	8– 9	58's	10	8·1
25·5–29·0	56's	11	8·9	6– 7	56's	1	0·8

The range and assigned qualities based on crimps per inch and fibre thickness are those established for South African commercial wools (Duerden, 1929; Duerden and Bosman, 1929), where the authors describe an analysis of grease wools procured from experienced woolmen and representative of the quality numbers recognised in wool buying practice. The standard limits for fibre thickness and for crimps per inch have since been used in the Wool Laboratory for comparing wools from Merino experiments. The frequency and percentage frequency for each quality are shown in Table 2.

Of 123 wool samples analysed 0·8 per cent. are 90's on crimping; 1·6 per cent. are 80's; 8·1 per cent. are 70's; 30·9 per cent. are 66's; 36·6 per cent. are 64's; 13·0 per cent. are 60's; 8·1 per cent. are 58's; and 0·8 per cent. are 56's. The largest percentage, namely, 6·75 per cent., of stud rams in the Union are 64-66's, or a medium quality on crimping (Schuurman, 1929). This method of estimating the quality number of wool is frequently made use of by Merino breeders and woolmen.

In Table 2 is also given the frequency distribution of qualities as based on fibre thickness. In this case 0·8 per cent. are 90's; 5·7 per cent. are 70's; 5·7 per cent. are 66's; 14·6 per cent. are 64's; 24·4 per cent. are 60's; 39·8 per cent. are 58's; and 8·9 per cent. are 56's. The largest proportion, i.e. 64·2 per cent. of the Union's stud rams are 58's and 60's when based on fibre thickness, and for commercial purposes would be classed as strong wool.*

It is thus shown that 67·5 per cent. of stud ram wools are of a medium quality on crimps, while on fibre thickness 64·2 per cent. are a strong quality. These facts are expressed graphically in Figure 1, where curve A represents the distribution of the qualities based on crimps and curve B the distribution of qualities on thickness. If there were agreement between the quality number on crimps and that on thickness, the Modes of A and B would coincide. However, the Mode of A is at 64's and that of B at 58's, indicating that the larger percentage of the samples is 64's on crimping and 58's on fibre thickness.

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* The terms "fine," "medium" and "strong" wool are employed as used in South Africa for grading Merino wool (Schuurman, 1929).

A closer analysis of Table 1 is given in Table 3, where a comparison is made between the relationship ot the quality number on crimps and that on thickness. Columns one and two indicate the quality numbers on crimps and on thickness respectively, while the third column gives the frequency. The fourth column indicates the relationship between the quality on thickness and that on crimps, Table 3 is summarised as follows:—

Table 3.

Quality on Crimps.	Quality on Thickness.	Frequency.	Quality on Thickness in Relation to Quality on Crimps.
90's	90's	1	Agreement.
80's	70's	1	Coarser by 1 qual.
„	64's	1	„ „ 3 „
70's	66's	3	„ „ 1 „
„	64's	3	„ „ 2 „
„	60's	3	„ „ 3 „
„	56's	1	„ „ 4 „
66's	70's	4	Finer by 1 „
„	66's	1	Agreement.
„	64's	10	Coarser by 1 qual.
„	60's	8	„ „ 2 „
„	58's	14	„ „ 3 „
„	56's	1	„ „ 4 „
64's	70's	1	Finer by 2 „
„	66's	3	„ „ 1 „
„	64's	4	Agreement.
„	60's	10	Coarser by 1 qual.
„	58's	23	„ „ 2 „
„	56's	4	„ „ 3 „
60's	70's	1	Finer by 3 „
„	60s'	7	Agreement.
„	58's	6	Coarser by 1 qual.
„	56's	2	„ „ 2 „
58's	60's	2	Finer by 1 „
„	58's	6	Agreement.
„	56's	2	Coarser by 1 qual.
56's	56's	1	Agreement.

1 or 0·8 per cent. of samples are finer on thickness than crimps indicate by 3 qual.

1 or 0·8 per cent. of samples are finer on thickness than crimps indicate by 2 qual.

9 or 7·3 per cent. of samples are finer on thickness than crimps indicate by 1 qual.

20 or 16·3 per cent. show agreement between quality on crimps and that on thickness.

32 or 26·0 per cent. of samples are coarser on thickness than crimps indicate by 1 qual.

36 or 29·3 per cent. of samples are coarser on thickness than crimps indicate by 2 qual.

22 or 17·9 per cent. of samples are coarser on thickness than crimps indicate by 3 qual.

22 or 1·6 per cent. of samples are coarser on thickness than crimps indicate by 4 qual.

It is thus shown that:

8·9 per cent. of the samples are finer in fibre thickness than the crimps indicate.

16·3 per cent. show an agreement between quality on thickness and quality on crimps.

74·8 are coarser on fibre thickness than the crimps indicate.

An analysis of commercial wool samples, where standard limits for crimps and fibre thickness were established (Duerden and Bosman, 1929) showed that 75 per cent. of the samples analysed were in agreement with the standards. In the present study where these same standards were taken as a basis and wool from stud rams was considered, there is a 16·3 per cent. agreement, while 74·8 per cent. of the samples are coarser on fibre thickness than the crimps indicate, and 8·9 per cent. are finer. These facts are of significance to the sheep breeder and to the woolman, in that it is shown that ram's wool in three cases out of four is coarser in fibre thickness than the crimps indicate. In other words, when quality estimation is based on crimps, an allowance should be made for a coarser fibre thickness in three cases out of four.

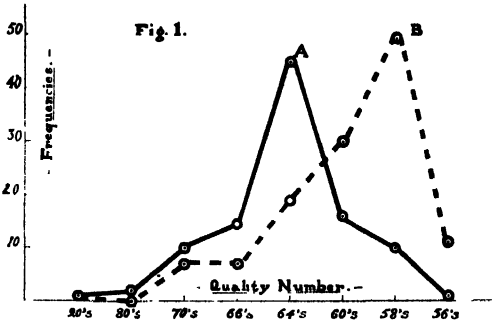
Fig. 1.

C.—VARIABILITY.

The mean fibre thickness of a sample forms a basis for classifying a wool into its quality number, but the mean does not indicate the degree of fibre variability which is influenced by the number of thickness classes, the range of these and the frequency distribution.

The statistical treatment of fibre measurements has received the attention of various research workers, Henseler (1926), Probst (1926), Roberts (1930). S. G. Barker (1931) states that: "It is not only the average fineness of a sample and its frequency distribution, but also its coefficient of variation, expressing as it does the variation within the staple, that is of supreme importance." The degree of fibre variability of wool is a factor of significance from the wool manufacturer's point of view. To what extent fibre variability of stud rams is of importance from a genetic aspect is still being investigated. Expressions of variability, namely Standard Deviation and Coefficient of Variability, are useful when fibre uniformity are studied.

The Standard Deviation bears a close relationship to the form or shape of the distribution curve. It expresses in absolute terms the degree of scatter or dispersion of the variates. It also gives an indication of fibre purity of any one sample and roughly three times the Standard Deviation on either side of the mean will include all the variates.

Examples of thickness distribution curves of rams' wools are given in Figures 2, 3 and 4.

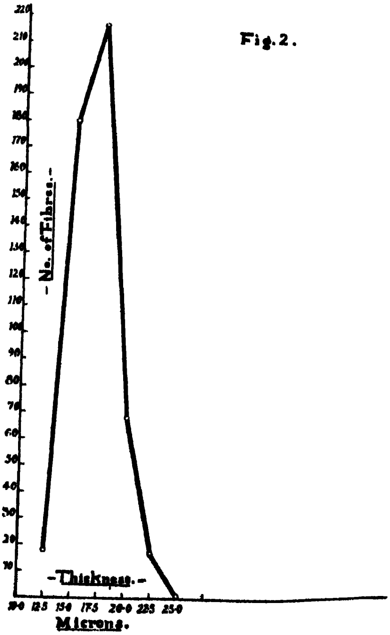

Fig. 2.

Fig. 2 is the curve of sample 39/11 and shows a uniform distribution with a low standard deviation of 0·863. The Mode is 17·5μ with a frequency of 216, so that 43 per cent. of the fibres measure 17·5μ. The thickness ranges from 12·5μ to 25μ with 6 class intervals, and a mean of 16·96μ, a 90's wool.

Fig. 3 is the curve of sample 2/1. The standard deviation is 1·277. The fibre thickness ranges from 12·5μ to 30μ with 8 class intervals, and the Mode at 20·0μ with a frequency of 180 or 26 per cent. of fibres measuring 20·0μ. The mean is 20·03μ, a 64's wool.

Fig. 4 is the curve of sample 18/1, which shows a more variable distribution with a standard deviation of 1·827, and a range from 12·5μ to 42·5μ. The Mode is at 25·0μ with a frequency of 127 or 25 per cent. of fibres of 25·0μ. The mean is at 24·51μ, a 58's wool.

The standard deviation of the rams' wool ranges from 0·863 to 2·094. Although a useful constant for variability, its utility for comparative purposes is restricted and wools of differing mean thicknesses such as in Table 1 are also compared on a relative basis by the coefficient of variability, as the latter expresses the Standard Deviation as a percentage of the mean. The coefficients of variability of rams' wool ranges from 5·1 per cent. to 8·7 per cent. An analysis of the frequencies of the coefficients of variability is given in Table 4.

TABLE 4.

Range of Coefficient of Variability as Percent.	Frequency.	Percentage Frequency.
5·1–5·5	6	4·9
5·6–6·0	24	19·5
6·1–6·5	29	23·6
6·6–7·0	24	19·5
7·1–7·5	25	20·3
7·6–8·0	12	9·8
8·1–8·7	3	2·4

The largest portion of the South African Merino stud sires, namely, 82·9 per cent. have wool which ranges from 5·6 per cent. to 7·5 per cent. as regards coefficient of variability, 4·9 per cent. have a lower coefficient of variability, namely, from 5·1 per cent. to 5·5 per cent. and are relatively more uniform; while 12·2 per cent. are higher in this respect, namely, from 7·6 per cent. to 8·7 per cent. and are more variable.

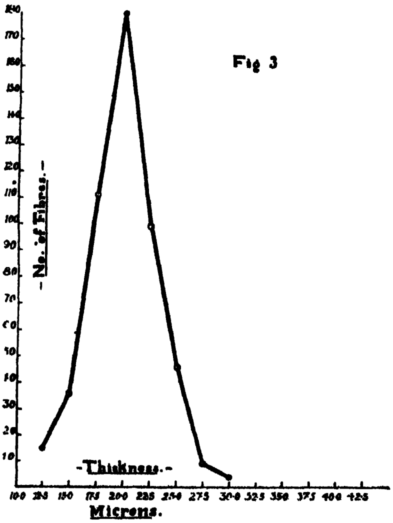

Fig 3

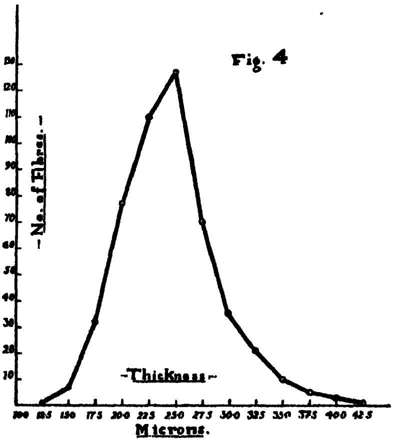

Fig. 4

D.—Correlation.

Correlations between wool attributes are useful to the practical man as conclusions can often be formed by assuming relationships between characteristics. The coefficients of correlation in the present study are given in Table 5.

Table 5.

	Fibre Thickness.	Crimps per Inch.	Stand. Dev.	Coeff. of Var.
Fibre Thickness	—	— ·426 ± ·0498 Def. Neg. Corr.	+ ·677 ± ·0327 Def. Pos. Corr.	+ ·020 ± ·0608 No. Corr.
Crimps per Inch	— ·426 ± ·0498 Def. Neg. Corr.	—	— ·2596 ± ·0567 No. Corr.	— ·0218 ± ·061 No. Corr.
Stand. Dev....	+ ·677 + ·0327 Def. Pos. Corr.	— ·2596 ± ·0567 No. Corr.	—	+ ·0659 ± ·0671 No. Corr.
Coeff. of Var...	+ ·020 ± ·0608 No. Corr.	— ·0218 ± ·061 No. Corr.	+ ·0659 ± ·0671 No. Corr.	—

Between fibre thickness and crimps per inch the value of −0·426÷0·0498 indicates a definite negative correlation, although not a high one. This means that in general the more crimps per inch there are the finer is the wool.

As regards fibre thickness and standard deviation, the coefficient of correlation of +0·677± ·0327 shows a definite positive correlation or, the coarser the wool, the higher the standard deviation.

There is no definite correlation between fibre thickness and coefficient of variability as the coefficient of correlation of + ·020± ·0608 indicates.

Likewise the coefficient of correlation −0·2596±0·0567 between crimps per inch and standard deviation indicates no definite correlation. Between the standard deviation and coefficient of variability the value +0·0659±0·0671 shows no definite correlation.

DISCUSSION.

A 1931 survey of wool from stud rams in the Union demonstrated the existence of a dominant type. On crimping, 67·5 per cent. of the wools are 64/66's, or a medium quality, and from a show and sale ring point of view this percentage would be regarded as medium wool. On fibre thickness 64·2 per cent. of the rams have a 58/60's wool or a strong quality.

It is also shown that in three cases out of four ram's wool is coarser in fibre thickness than the crimps indicate. This fact is sufficient reason for advising the wool farmer to separate stud ram wool from his general fleece lines. It is also evident that wool from stud rams requires different standards of crimping and fibre thickness from those established for commercial flock wools. From the manufacturer's point of view fibre thickness plays a more important rôle in spinning that do crimps as such; the wool buyer's valuation in respect of fineness being based more on fibre thickness than on crimping.

The stud rams concerned in the study are valuable animals from the owners' point of view and are consequently better cared for than flock sheep. It was suggested that the fact could have influenced their wool characteristics, but a comparison with certain well-fed experimental sheep at Grootfontein showed that this was not the case as the latter wools agreed with the standards of commercial flock wool.

As regards correlation between fibre thickness and crimps per inch, the coefficient of correlation of $-0 \cdot 426 \pm 0 \cdot 0498$ although definite is not a high one. This relationship is established on wools well-grown and from shoulder regions away from skin folds. The correlation does not appear so definite when sampling includes wools on skin folds (Reimers and Swart, 1929), Bosman, 1933) (1) or droughty wools (Duerden, 1929; Duerden and Bosman, :1929).

Hultz and Paschal (1930) in "Wool studies with Rambouillet Sheep," found an insufficient correlation between crimp and fibre thickness to warrant reliability being placed on crimp as an indication to fibre thickness.

Duerden and Bosman (1929) found a sufficient agreement between crimp and thickness of commercial wools well-grown taken away from skin folds to warrant estimations for quality number being based on crimps. From the foregoing study it appears that ram wools constitute an anomaly.

The South African wool clip which includes droughty wools, fold wool, ram wools and other types will only show agreement on crimps and fibre thickness to a limited degree, and the quality number of the limitations will be treated on the merits of fibre thickness. Quality number based on fibre thickness by hand and eye methods is more difficult to estimate than that based on crimps especially in the finer qualities, 80's and above, and there are instances where wool buying firms have resorted to laboratory methods for estimating fibre thickness for the finer qualities.

The variability or uniformity of fibre distribution is also a factor influencing the spinning of wool. The standard deviation is a useful expression for variability. Where the analysis of all the ram's wool is based on 500 fibres, the distribution curve is valuable for indicating the fibre scatter within the staple. The standard deviations range from the lowest to the highest in harmony with an increase in fibre thickness from the finest to the coarsest wool.

The largest number of fibres of one thickness in any one sample was 218 out of 500 or 44 per cent. The least was 100 or 20 per cent. The largest portion of the samples, namely 65·8 per cent. have a fibre uniformity of 26·30 per cent. The average for fibre uniformity of stud rams is approximately 30 per cent. It is also of interest that about 15 per cent. of stud rams in the Union have a fibre uniformity of 40 per cent.

The coefficient of correlation of $+0·677 \pm ·0327$ between thickness and standard deviation shows a definite correlation, indicating that fine wools have greater fibre uniformity within the staple than coarse wools. This fact is also evident in the fibre thickness standards of Duerden (1929) and of Dantzer and Roehrich (1928), where the thickness limits for the coarser qualities are wider than those for the finer wools.

In an analysis of " Fibre Lengths, Thickness and Qualities in a Single Wool Staple," (Duerden and Bosman, 1931), it was shown that " a wool very variable in length will also be very variable in thickness, or a wool uniform in length will be uniform in thickness. In striving for the uniformity of the one, the breeder will tend to attain uniformity of the other."

From the foregoing it also follows that fine wool will have a higher percentage fibre uniformity within the staple than coarse wool, both as regards fibre thickness and fibre length. Comparisons based on standard deviation should be limited to within the different grades; fine wools with fine and coarse wools with coarse.

The coefficient of variability expresses the standard deviation as a percentage of the mean thickness and is shown to vary from 5·1 per cent. to 9·0 per cent. This constant is limited in its usefulness for Merino wool as in itself it does not indicate the degree of scatter or the degree of fibre uniformity within the staple. It is, therefore, suggested to use both the standard deviation and coefficient of variability as measures of variability in wool studies.

It will be of interest to wool producers to know how far facts established for stud rams are showing themselves in the Union's wool clips, as it is the stud rams that breed flock rams and these in turn influence the commercial clips. The stud rams of 1931 will have lambs suitable for use in 1933 and the progeny of these when used for flock improvement purposes can only reflect their characteristics in the Union's wool clip from 1934.

ACKNOWLEDGMENT.

The authors wish to express their thanks to the Merino stud breeders of the Union who so kindly supplied them with wool samples and relevant information.

SUMMARY AND CONCLUSIONS.

1. The wool characteristics of Merino stud rams are of significance to the wool industry of the Union since the progeny of these rams influence the commercial wool clips.

2. The mean fibre thickness of wools from stud rams ranges from 16·96μ to 27·62μ, which includes all the Merino qualities from 90's to 60's, as well as the coarser ones of 58's and 56's.

3. The frequencies of the qualities based on fibre thickness indicate that 0·8 per cent. are of 90's quality; 5·7 per cent. are 70's; 5·7 per cent. are 66's; 14·6 per cent. are 64's; 24·4 per cent. are 60's; 39·8 per cent. are 58's; 8·9 per cent. are 56's. Thus on fibre thickness, 64·2 per cent. of the stud rams are of 58's to 60's, which would be regarded commercially as strong wool.

4. The crimps range from 21 per inch to 6 per inch, which likewise includes all the Merino qualities and coarser ones of 58's to 56's. The frequencies show that: 0·8 per cent. are of 90's; 1·8 per cent. are 80's; 8·1 per cent. are 70's; 30·9 per cent. are 66's; 36·6 per cent. are 64's; 13 per cent. are 60's; 8·1 per cent. are 58's and 0·8 per cent. are 56's. On crimping, therefore, 67·5 per cent. produce qualities 64's to 66's, commercially regarded as a medium wool.

5. Rams' wool in three cases out of four is coarser in fibre thickness than the crimps indicate. This result is compared with that obtained in the establishment of standards from commercial flock wool where a 75 per cent. agreement between standards of crimps and thickness was obtained.

6. Between fibre thickness and crimping there is a coefficient of correlation of $-0·426 \pm ·0498$, which is a definite one, though not high. In general the more crimps per inch there are the finer is the wool.

7. The standard deviation of fibre thickness ranges from 0·863 to 2·094. In the most uniform sample 44 per cent. of the fibres are of one thickness. The standard deviation is 0·863. The least uniform sample has 20 per cent. fibre uniformity, and a standard deviation of 2·09.

8. The average fibre uniformity for stud rams is approximately 30 per cent. 15 per cent. of stud rams in the Union have a fibre uniformity of 40 per cent., and are relatively very uniform.

9. Fine wools have a higher fibre uniformity than coarse wools. The coefficient of correlation between mean thickness and standard deviation is $+0·677 \pm 0·0329$.

10. The coefficient of variability varies from 5·1 to 8·7 per cent. 82·9 per cent. of the samples have this value from 5·6 per cent. to 7·5 per cent., 12·2 per cent. have a range from 7·6 per cent. to 8·7 per cent., and 4·9 per cent. are relatively very uniform with a value of 5·1 per cent. to 5·5 per cent.

11. There is no coefficient of correlation between coefficient of variability and fibre thickness as is shown by the value $0·020 \pm 0·0608$.

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Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 1, Number 2, 1933.

The Effect of Barley, Millet (*'n Yati-Pennisetum* sp.) and Lucerne Meal in Bacon Production.

By G. N. MURRAY, B.Sc.Agric., D. J. SCHUTTE, M.Sc.Agric., and J. A. DU PLESSIS, B.Sc.Agric., Research Officers, Onderstepoort.

INTRODUCTION.

The results of previous experiments (Romyn and others, 1930), have shown that from the producer's point of view a ration consisting of 90 per cent. maize and 10 per cent. meat meal proved most economical for bacon pigs. Baconers fed on this ration, however, tended to produce soft fat, and in some cases the back fat was too thick. The present trial was planned with the object of obtaining more information on the influence of a ration in which maize is partly substituted by a millet. Barley meal was again used to compare with work previously done (Schutte and Murray, 1931). Meat meal being an expensive protein ingredient, half the meat meal was substituted by 15 per cent. lucerne meal. Three rations was thus compared with the standard maize-meat meal ration. This trial was carried out at the School of Agriculture and Experiment Station, Potchefstroom.

MATERIALS AND METHODS.

On the 8th April, 1932, 40 pure bred Large White weaners were divided into four equal lots. The following rations were fed to the four lots, the proportions being by weight:—

Lot I.—Ground maize 90, meat meal 10.

Lot II.—Ground maize 80, meat meal 5, lucerne meal 15.

Lot III.—Ground maize 45, ground millet 45, meat meal 10.

Lot IV.—Ground maize 45, ground barley 45, meat meal 10.

To all the rations 5 per cent. of a mineral mixture consisting of 4 parts bone meal and 1 part salt was added. Equal quantities of green feed were given daily to each lot. The meal rations were fed twice daily in the form of a thick slop, the pigs receiving as much as they could clean up in about 30 minutes. The pigs were kept in dry ¼-acre paddocks.

Individual weights were taken every week in the morning a few hours after the pigs had their food. When the proper weights had been reached the pigs were railed to the Farmers' Co-operative Bacon Factory, Estcourt, Natal, a distance of 415 miles, where they were killed a day after arrival and detailed data recorded on all carcases.

The methods employed in the grading of the carcases were described by Romyn and others (1930).

RESULTS.

In Table 1 the average weights, daily gains, and feed consumption of the pigs in the four lots are given:—

TABLE 1.

	Lot I.	Lot II.	Lot III.	Lot IV.
Number of pigs	10	10	10	10
Initial age—days	78·9	79·0	77·9	78·0
Final age—days	177·1	174·9	167·3	173·6
Days in experiment	98·2	95·9	89·4	95·6
Initial weight—lb	57·6	57·3	48·7	49·0
Final weight—lb	201·8	200·4	199·4	204·2
Total gain—lb	144·2	143·1	150·7	155·2
Average daily gain—lb	1·47	1·49	1·69	1·62
Total concentrates consumed—lb	4,570	4,700	4,679	5,086
Average daily food intake per pig—lb	4·7	4·9	5·2	5·3
Food consumed per 100-lb. gain—lb	316·9	328·4	310·5	327·7

The average weights and measurements and the grading of the carcases are given in Table 2.

TABLE 2.

	Lot I.	Lot II.	Lot III.	Lot IV.
Farm live weight—lb.	201·8	200·4	199·4	204·2
Factory live weight—lb.	178·7	175·8	178·8	180·7
Percentage of farm weight	88·6	87·6	89·6	88·5
Dressed weight—lb.	150·8	145·2	150·5	150·0
* Dressing percentage	74·7	72·4	75·4	73·5
Curing weight—lb.	114·5	115·8	120·3	113·9
* Curing percentage	56·7	57.7	60·3	55·8
Thickness of back fat—				
Shoulder—cm.	5·5	5·3	5·5	5·7
Flank—cm.	2·7	2·6	3·1	2·8
Loin—cm.	3·4	3·2	3·7	3·4
Average—cm.	3·9	3·7	4·0	4·0
† Evenness—per cent.	49·5	49·4	55·8	50·0
Thickness of belly—cm.	2·9	2·8	3·2	3·3
Length of side—cm.	76·8	76·2	75·0	75·1
Depth of side—cm.	39·5	38·9	40·5	40·0
Circumference of ham—cm.	59·0	58·6	59·6	58·7
Length of ham—cm.	35·8	35·7	35·0	35·5
Ratio (C/L x 100)—per cent.	164·9	164·3	170·5	165·6
Texture of back fat				
Firm—per cent.	90	90	100	100
Medium firm—per cent.	10	10	—	-
Medium soft—per cent.		—	—	—
Soft—per cent.	—	—	—	—
Average refractive index at 40° C.	1·4592	1·4593	1·4589	1·4588
Points awarded for—				
Length of side—per cent.	82	78	75	79
Thickness of belly—per cent.	70	60	82	80
Proportion of lean meat—per cent.	81	81	82	80
Proportion of fat—per cent.	79	81	75	78
Uniformity of fat—per cent.	84	82	81	82
Marbling of lean meat—per cent.	72	72	72	74
Plumpness of ham—per cent.	79	73	82	80
Grading of sides—				
No. 1 lean sizeable—per cent.	80	70	70	80
No. 2 lean sizeable—per cent.	-	30	10	10
No. 1 medium—per cent.	20	-	20	10

* The dressed and curing weights are expressed as percentages of the farm live weight.

† The thickness of the back fat at the flank (thinnest measurement) is expressed as a percentage of the thickness at the shoulder (thickest measurement).

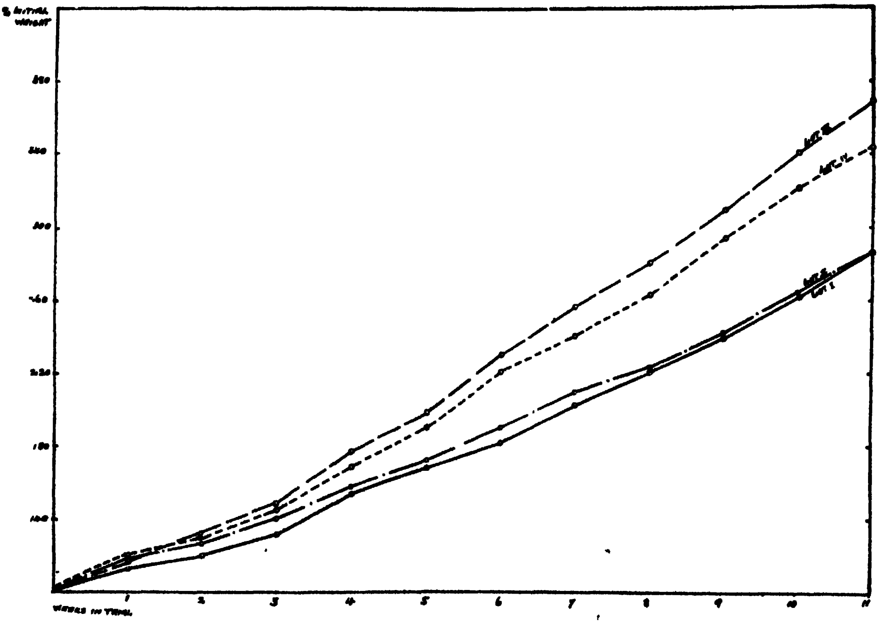
DISCUSSION OF RESULTS.

The pigs in the four lots made very good gains, those in Lots III and IV being somewhat better than those in the first two lots, which are practically the same. The pigs in the two lots that made the best gains, however, also consumed the largest amounts of food per day. Since the average initial weights of Lots III and IV were below that of the first two lots, the initial weights were taken as 100, and the subsequent weekly weights expressed as percentages thereof and the result is shown in diagram I. Lot I (maize-meat meal) remained behind from the start, whereas Lot II (maize, lucerne-meat meal) kept fairly close to Lots III and IV for about three weeks and then remained about the same as Lot I for the rest of the trial. From the seventh week the difference between Lots III and IV did not change much.

Throughout the trial the pigs were healthy and had excellent appetites, which is also indicated by the average daily feed consumed per pig during the experimental period. There is not much difference between the four lots as regards the food consumed per unit gain.

The carcase weights and measurements do not show large differences and in most cases these are small and insignificant. The lucerne meal lot (II) has the lowest dressing percentage, but also the thinnest back fat. The pigs in this lot also have the thinnest bellies with those in Lot I only slightly thicker. The standard errors have been calculated for the average lengths of Lots I, III and IV to see whether the differences which were obtained are significant. The results are: Lot I, 76·8 ± 0·95 cm.; Lot III, 75 ± 0·67 cm.; Lot IV, 75·1 ± 0·69 cm.; and the difference between Lots I and III is 1·8 ± 1·2 cm. and between Lots I and IV 1·7 ± 1·2 cm., both differences, threefore, being insignificant, so that the food had no direct or indirect effect on the length of the pigs. Schutte and Murray (1931), however, maintained " that barley exercises a favourable influence on length of side ", but no standard errors were given and at the same time the barley fed lots were 9·3 and 10·4 lb. heavier than the maize-meat meal lot. The following are the standard errors of the mean lengths which they obtained: Lot I (maize-meat meal), 28·9 ± 0·29 in.; Lot II (45 maize, 45 barley, 10 meat meal), 29·6 ± 0·23 in.; Lot III (70 barley, 20 maize, 10 meat meal), 29·5 ± 0·25 in.; and the difference between Lots I and II is 0·7 ± 0·36

DIAGRAM I.—*Relative rates of growth of the pigs in the four lots.*



in., and between Lots I and III is $0{\cdot}6 \pm 0{\cdot}38$ in. In spite, therefore, that Lots II and III were heavier than Lot I, the differences were insignificant. The depth of the sides are in the same order as the thickness of the back fat, the fattest group also having the deepest sides, which agrees with Murray's results (1933). To get a measure of the plumpness of the ham the circumference has been expressed as a percentage of the length. The points awarded for the plumpness of the ham according to sight, follow the same sequence as regards the four lots, although the differences are not quite the same.

The firmness of the back fat, as determined by the refractive index, of all the lots is very good, that of Lots III and IV being firmer than that of Lots I and II. The average refractive index of Lot I (1·4592) is lower than the average (1·4595-1·4603) of all previous trials where the same ration was used. The same is the case with the barley lot (1·4588), since the averages of previous trials ranged from 1·4591-1·4595. In the previous trials the pigs that were used were the crosses of Large White, Large Black and Tamworth breeds. As reported by Kelly (1932) there is a difference in the firmness of the fat between different breeds and this may also be the case between types of the same breed. This aspect is very important for a country like South Africa, where the staple pig food consists of maize.

In spite of the rapid gains, the grading of the carcases was very good. The carcases were also very uniform. No lot has less than 70 per cent. No. 1 lean sizeable carcases. Lot II has the largest percentage of inferior carcases and this was caused by the thin bellies of these carcases. The analysis made by Murray (1933) showed a positive correlation between rate of gain and thickness of back fat and that when pigs made very rapid gains the grading depreciated. The work was done with cross-bred pigs of the three breeds mentioned above. In the present trial the rapid gains did not have the adverse effect on the grading which one would have expected. In the mentioned analysis the optimum rate of gain for baconers appeared to be from 1·2 to 1·49 lb. per pig per day after the age of 10 weeks. It would therefore appear that for different types of pigs the optimum rate of gain, to get the best grading, is different.

Since there were only differences in the gains made by the pigs and no difference in carcase quality caused by the food, it will, therefore, depend on the prices whether one can use such ingredients as lucerne, millet or barley. As in previous trials the maize-meat meal ration was again the cheapest, being 2·37 pence per pound gain. The ration of Lot II was 2·41, Lot III 2·94 and Lot IV 2·66 pence per pound gain in live weight when the contract prices are taken which ruled when the trial was started. The contract prices per 100 lb. food were: maize 5s. 4d., millet 9s., barley 6s. 6d., meat meal 11s., lucerne meal 6s. 6d. When prices as given above are ruling for the foodstuffs then the best return in bacon production is realised by using the cheap maize-meat meal ration.

SUMMARY.

Three rations were compared with the standard maize-meat meal ration when fed to baconers. Excellent gains were made on all the rations, the pigs on the millet and barley rations, however, making

the best gains, but these lots also consumed the largest amounts of food daily. The difference in rate of growth slightly influenced the degree of fatness of the pigs, otherwise there were no significant differences in the carcase measurements of the pigs in the four lots. The fat appeared to be firmer than that of pigs of previous trials which had similar rations. Type of pig may have caused this difference. The grading was good and the rate of gain had no adverse influence, so that it appears that different types of pigs have different optimum growth rates for the production of first grade bacon. The standard maize-meat meal ration again proved to be the most economical in bacon production.

ACKNOWLEDGMENT.

Grateful acknowledgment is due to Mr. D. J. R. van Wyk, M.Sc., of the Division of Chemistry, for his technical assistance in connection with the refractive index determinations, and to the management of the Farmers' Co-operative Bacon Factory, Estcourt, for placing the facilities which their factory affords to the disposal of this Division for experimental purposes.

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Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 1, Number 2, 1933.

Studies in Native Animal Husbandry (1).

(6) A Note on Ovambo Cattle.

By J. W. GROENEWALD, M.Sc.(Agric.), and H. H. CURSON, F.R.C.V.S., Dr.Med.Vet., Research Officers, Onderstepoort.

INTRODUCTION.

It having been emphasized recently by Quinlan, Bisschop and Curson (1932) that the indigenous cattle of South Africa had not received the attention they deserved, we venture to record a few observations made on several head of Ovambo cows introduced from South-West Africa Protectorate in 1929. It should be noted that Ovamboland, being situated without the Police Zone, is probably the least accessible territory south of the Zambesi River. One would expect, therefore, that Ovambo cattle have been less disturbed by the march of civilisation than elsewhere in the sub-continent.

Until the arrival of the cattle referred to above, the only material available consisted of two skulls (see Figs. 1 and 2), from a bull and cow respectively, received from Lieut. C. H. Hahn, Ondonga, Ovamboland, in 1922.

VIEWS REGARDING CATTLE IN SOUTH-WEST AFRICA.

But few references to Ovambo cattle are available. Molhuysen (1911), however, has collected quite a number, the most useful being those of Schinz (1891) and Hermann (1902). Schinz states that prior to European occupation there were in South-West Africa two types of cattle, the Ovambo in the north and the Herero or Damara in the south. The latter he compares with the Hottentot cattle to be found apparently still farther south. According to Hermann, as a result of the northerly migration of the Rhehoboth Bastards from the Cape Colony a change in type occurred, resulting in the production of what is to-day the Nama, a cross between the indigenous and introduced, i.e. European, types. The only reference Ostertag (1912) makes to the Ovambo beast is that it is one of the four(²) indigenous types of South West African cattle. He gives, however, an excellent picture of Ovambo oxen (Fig. 10, p. 32). In 1914 Schlettwein's description of farming conditions in South West Africa Protectorate appeared as a second edition, and reference will be made to this in discussing the observations of the next worker.

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(1) The following studies have appeared:—(i) Notes on the Wankonde. *Jl. S. Afr. Vet. Med. Assn.* I (4). (ii) Proposed plan of investigation, *Id.* II (2). (iii) Native milking-pails. In the press: (iv) Bantu and cattle in the Northern Transvaal, *Jl. S. Afr. Vet. Med. Assn.*, II (2). (v) Indigenous cattle in the Transkeian Territories, *Id.* III (4). (vi) This paper. (vii) Makalanga cattle—a representative described. *This Journal.* (viii) The domesticated animals of Pre-European S. Africa, *Jl. S.A.V.M.A.*, IV (2).

In 1928 Dr. G. Schmid, Government Veterinary Officer, Omaruru, submitted an interesting report (S.V.O., Windhoek, Minute A/2 of 16/8/28). The portion dealing with the Ovambo is reproduced *in extenso* as follows:—

"The Ovambo cattle: This third type was chiefly restricted to the north of the territory and was mostly in possession of the Ovambo tribes. This type is very similar to the type found in the inner parts of equatorial Africa, and is also an indigenous African breed.

"The animals are small but well proportioned, with fine bones of great density, small head and long and lyre-shaped horns. The predominant colour is a light or dark grey-brown (game colour). The Ovambo cattle have acquired great resistance against diseases, and I found that many of them did not react on lung-sickness vaccination, because they apparently possessed a natural immunity against the disease. The milk production is low but the beef is of a fine texture. These cattle were much appreciated by the early European farmers of the north as a good foundation stock, on which to build, which gave very satisfactory results when crossed with imported breeds, especially for beef production. An estimable attribute in this type is its great adaptability to different climatic conditions; for even in times of drought the Ovambo cattle keep their condition and fertility.

"There is no doubt that from this type, under better conditions and with some knowledge of breeding, a very good and hardy breed could be built up. The Ovambo tribes, however, are more an agricultural people and have not the interest in their cattle in comparison with the Hereros. I saw some very good specimens of this type on the Okavango River."

Schmid adds:—

"The devastations of Rinderpest, 1899-1900, and later of the native war, 1904-06, sadly depleted the herds of native cattle in South-West Africa. Rohrbach states: "After the war, 1906, the whole native stock was exterminated with the exception of about 3,000 cattle, which were distributed to the farmers. Also nearly all the cattle in possession of the old farmers were lost. Only the

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(²) The other are Damara, Bechuana and Nama. With regard to the last, Dr. Schmid writes that they are a cross chiefly of Afrikander and Friesland and belong to the coloureds of the south.

Rhehoboth Bastards saved about 600 female cattle. Consequently the herds of the farmers and newcomers had to be built up eventually almost entirely from imported cattle from the Cape and Europe. During 1906 to 1912 alone, about 15,000 cattle were imported from the Cape and Bechuanaland . . .

" . . . The material for this report has been partially collected from the following sources:—

1. Rohrbach, Deutsche Kolonialwirtschaft, I. Band, S.W. Afrika.
2. Schlettwein, Der Farmer in Deutsch Südwest Afrika.
3. Ostertag, Veterinärwesen und Fragen Der Tierzucht in D.S.W.A.

(Denkschrift über die Rinderzucht des D.S.W.A. Schutzgebiets, 1912) ".

The above remarks relating to the position after the German-Native campaign of 1904-06 probably do not refer to the Ovambo cattle, since being so far north they apparently escaped the ravages of war.

THE ONDERSTEPOORT HERD.

Six cows, later numbered from 3584-89, arrived at Onderstepoort on 20th June, 1929, having been entrained at Tsumeb. At Windhoek they were inoculated for anthrax (according to the export regulations) and then forwarded to Onderstepoort under quarantine conditions. Actually seven head were purchased by the Chief Agricultural Officer (Dr. P. v. d. H. Schreuder), but one beast died *en route*. The price paid was £4 per head.

On arrival at Onderstepoort the animals were naturally poor in condition, their weights ranging from 550-600 lb. They thus " felt " the colder winter of the Transvaal Middleveld more than otherwise. Their ages were approximately 10 years, but old cows had expressly been purchased in order to be surer of obtaining cattle of the pure Ovambo type. At first the cows refused food from a manger and water from a trough, but it was not long before they became accustomed to these receptacles of civilisation. Their behaviour, however, was never as quiet as that of European cattle, there always being a certain nervousness. The only ecto-parasites observed on their arrival were ticks, *R. evertsi* var. *albigeniculatus* being common beneath the root of the tail.

Although clearly of native stock, it was obvious the cattle represented a definite type. Regarding origin, too little is known of this aspect of African types, and to discuss the matter at this juncture would be most unprofitable. It is, however, remarkable that State officials (especially museum authorities) have manifested little or no interest in this important subject.

Since it was believed that the cows would be susceptible to tick-borne diseases, such as redwater, gallsickness and heartwater, appropriate precautions were taken. On 25/6/29 they were injected with

5 c.cm. of redwater and gallsickness vaccine, but in all cases the reactions were inconclusive. This is most surprising in view of the fact that western (Kalahari) cattle are believed to be susceptible to redwater and gallsickness. The attached chart is representative of the temperatures recorded over a period of two months:—

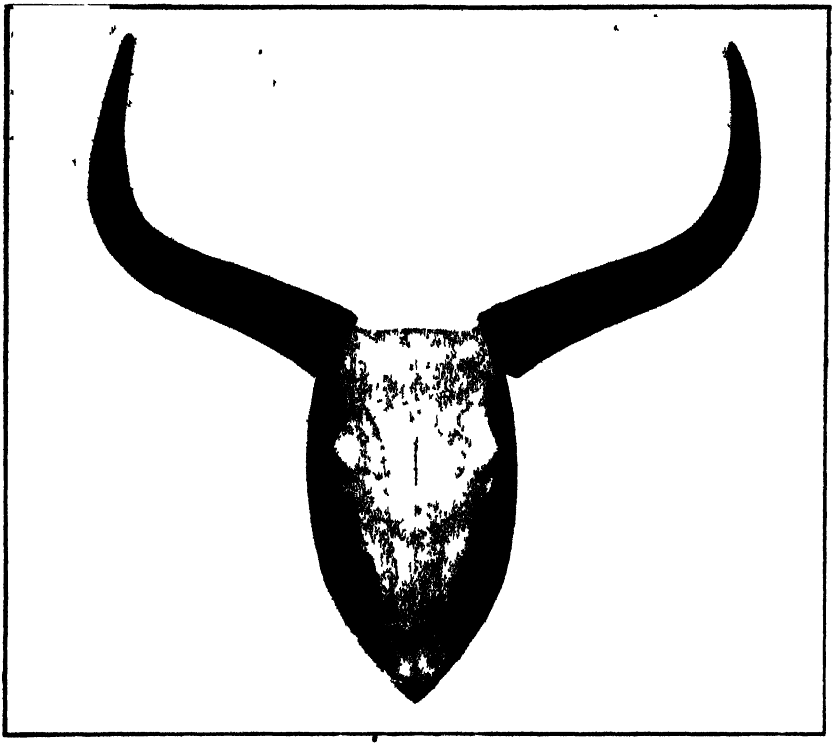
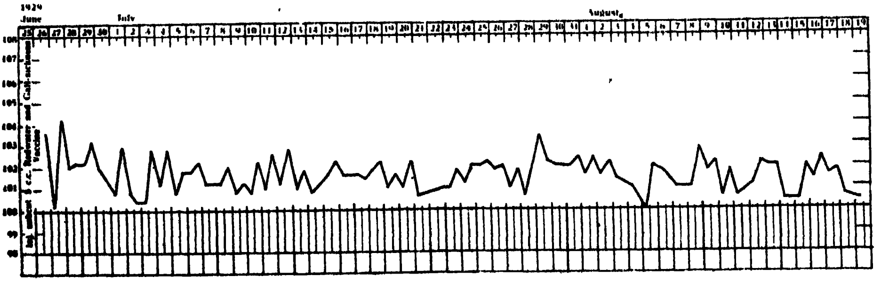


Fig. 1. Ovambo skull, bull.

Temperature Chart.—Ovambo Cow D.O.B. 3586.



While the horns, hump, dewlap, navel and sloping quarters are features of indigenous cattle as a whole, there is no doubt that the Ovambo is a product of its environment. Although Schlettwein states that the cows give generally more milk than the neighbouring Damara cattle, yet on account of its short sturdy build, it is for beef purposes that the type seems best suited. For grading with bulls of European breeds, the Ovambo would prove excellent foundation stock. For transport purposes the small size of the oxen would be a disadvantage when compared with the larger Damara.

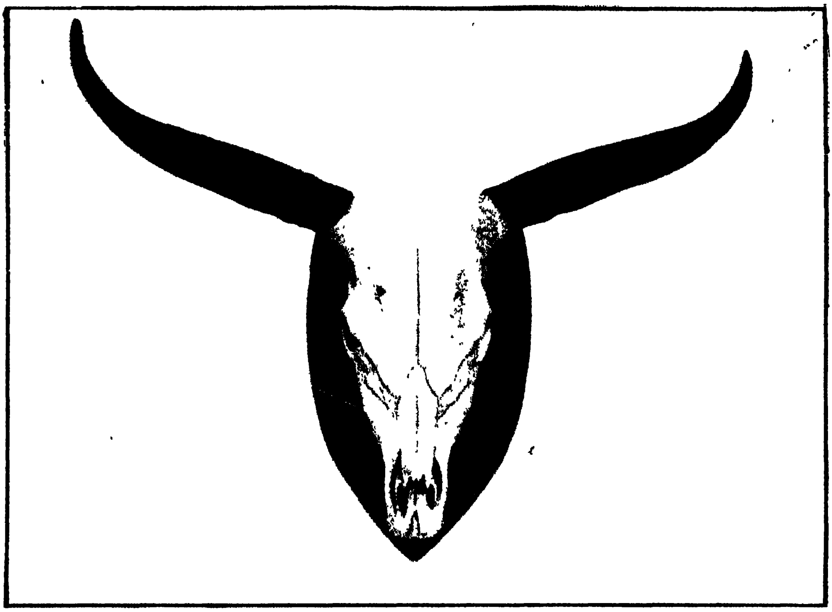


Fig. 2. Ovambo skull, cow.

Below is tabulated the information bearing on the cows referred to above: —

Cow.	Fate.	Progeny.	Born.	Fate.
3584	Killed 14.6.32 for Onderstepoort Museum. See milk record.	3661 male.... 4471 male ...	1.11.29 19.3.31	Died, icterus 6.4.31. Cast, type poor 17.8.31.
3585	Killed 13.2.30 for Transvaal Museum	—	—	—
3586	Used for milk records. Discharged 24/8/32	4301 female... 5010 male....	12.1.31 3.2.32	Cast, type poor 17.8.31. To reach maturity and then to be killed for Transvaal Museum.
3587	Used for milk records. Discharged 24.8.32	4654 female...	1.10.31	Cast 24.8.32.
3588	Killed 14.6.32 for Transvaal Museum	3666 male....	11.11.29	Contracted tuberculosis naturally. Castrated 13.6.32 and cast.
3589	Died 18.9.29. Skeleton in Onderstepoort Museum	—	—	—

Unfortunately, it was not possible to obtain an Ovambo bull from South-West Africa. Under the circumstances, a local native bull was used for service, except in the case of Bull 5010. Here the sire was Bull 3666, born of Cow 3588 five months after her arrival at Onderstepoort.

GENERAL CHARACTERISTICS OF THE OVAMBO COW.

HEAD.

Forehead: Broad and flat, i.e. not much dished.

Horns: Curved forward, sideward and upward.

Face: Fairly long with veins showing slightly.

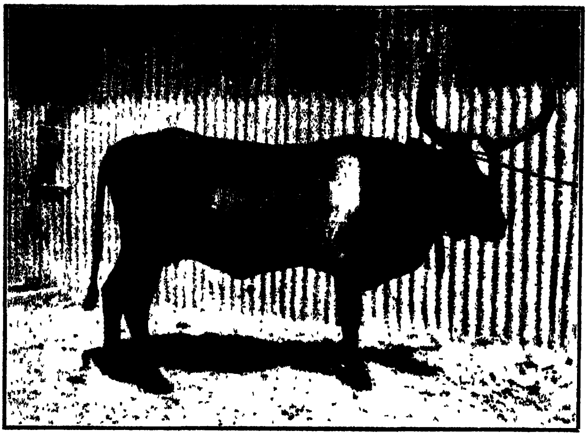


Fig. 3. Ovambo cow No. 3588.

Muzzle: Broad, strong and black.

Jaws: Strong and lips firm.

Eyes: Languid, with heavy fold of skin above.

Ears: Oval, pointed, stylish and alert.

NECK.

Short and broad, but neatly put on.

Dewlap: Prominent.

FOREQUARTERS.

Shoulders: Blend smoothly although slightly heavy.

Hump: Small and set well forward on withers.

Chest: Proportionately good depth but small, medium width between forelegs.

Brisket: Light.

Legs: Straight, light and fine boned.

Feet: Sharp and of medium size.

Joints: Firm and smooth.

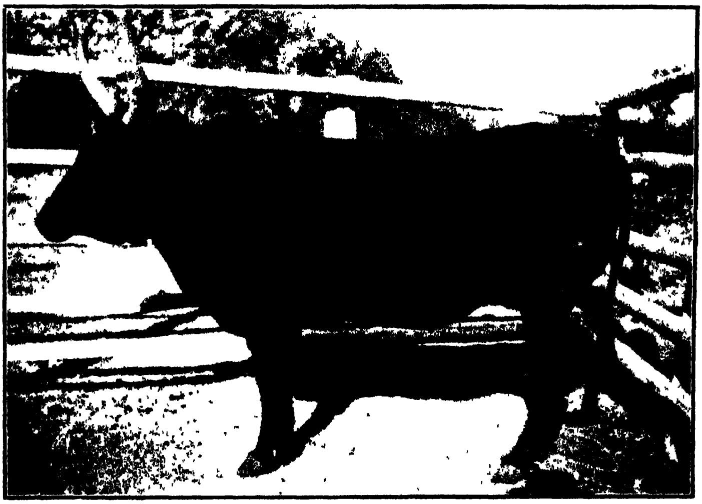


Fig. 4. Ovambo cow No. 3584.

BODY.

Back: Short and drooping.

Chine: Short and curved downward.

Loin: Medium, broad and sloping downward.

Ribs: Medium spring and not deep.

Abdomen: Round and shallow.

Navel: Characteristic loose skin fold.

Flank: Smooth, thin and of medium depth.

HINDQUARTERS.

Rump: Sloping from hook to pin bones as well as towards tail head, narrow, medium distance from pins to hooks.

Hooks: Not projecting much.

Pins: Pinched and low.

Thighs: Very small.

Tail: Long, but tail-setting prominent.

Legs: Straight, fine and clean cut.

Feet: Sharp and of medium size.

Shanks: Fine and smooth.

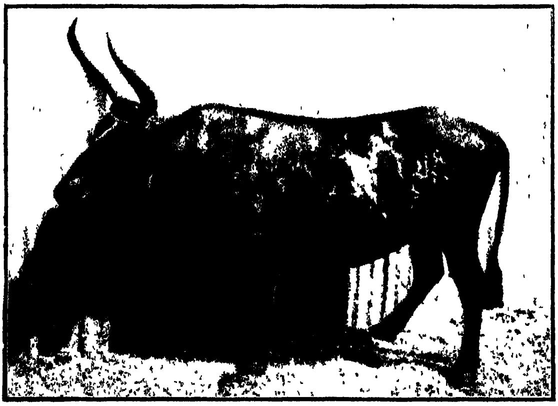


Fig. 5. Ovambo cow No. 3585.

UDDER.

Small, tight meaty bag, but well proportioned with ample loose folds stretching up behind.

Teats: Small and closely spaced.

Milk Veins: Prominent, tortuous and easily visible.

Escutcheon: Distinctly defined.

COLOUR.

Dun or black predominating.

COVERING.

Skin: Fine and elastic.

Hair: Soft and fine.

GENERAL CHARACTERISTICS OF THE OVAMBO BULL.

(Fig. 9.)

The bull differs from the cow mainly in exhibiting coarser horns, which follow an outward and upward curvature. The dewlap and brisket are heavier than in the cow, the hump is also larger and its well forward position gives the neck a thick-set throaty appearance.

The forequarters, body and hindquarters are, however, disappointingly small and light, being much like the cow in all respects and giving an effeminate impression.

Fig. 6. Ovambo cows Nos. 3587 and 3586.

These animals are extraordinarily nervous, are small and undersized, and show more beef than dairy qualities. Calves at birth weigh about 52 lb. and mature cows may be expected to average approximately 750 lb.

MILK OBSERVATIONS FROM SOME OVAMBO COWS.

The milk of the Onderstepoort herd was recorded and tested for fat and solids-not-fat at various stages during the different lactation periods. It was found impossible to draw more than a cupful of milk from a cow if the latter were milked without suckling the calf (see Fig. 10). The calves, too, seemed never to learn how to take milk from a pail. For these reasons the most satisfactory results could only be obtained by allowing the calves to suckle their dams and stay with them during the period of milking, a fact which may account for many irregularities in the tests, which are given in the following table:—

TABLE.

D.O.B. 3584. Calved 19.3.31.			
Date.	Milk.	Fat.	S-N-F.
	c.c.	%	%
2.4.31..	590	2·1	8·1
8.4.31..	550	1·4	8·7
15.4.31..	210	2·7	8·5
22.4.31..	500	2·5	8·7
29.4.31..	420	1·5	8·6
7.5.31..	300	1·7	8·8
13.5.31..	100	3·5	8·9
Average.	381	2·2	8·7

D.O.B. 3586. Calved 12.1.31.			
Date.	Milk.	Fat.	S-N-F.
	c.c.	%	%
2.4.31..	600	1·9	9·3
8.4.31..	620	1·9	9·5
15.4.31..	360	2·2	9·4
22.4.31..	410	2·3	9·0
29.4.31..	200	1·5	9·3
7.5.31..	410	1·4	8·5
13.5.31..	190	1·5	8·7
Average.	399	1·8	8·9

D.O.B. 3587. Calved 1.10.31.			
Date.	Milk.	Fat.	S-N-F.
	c.c.	%	%
8.10.31..	—	4·8	9·0
9.10.31..	—	5·2	8·5
10.10.31..	—	4·6	8·7
13.10.31..	—	6·8	8·3
14.10.31..	—	6·7	8·2
15.10.31..	—	7·2	7·4
16.10.31..	—	7·5	7·6
17.10.31..	—	5·2	8·4
20.10.31..	—	6·7	8·8
22.10.31..	—	5·1	8·6
23.10.31..	—	8·0	7·8
26.10.31..	—	5·0	9·8
10.3.32..	—	5·2	10·0
11.3.32..	—	3·9	10·0
8.5.32..	—	5·0	9·9
8.6.32..	—	4·6	10·0
Average.	—	5·7	8·7

D.O.B. 3586. Calved 3.2.32.			
Date.	Milk.	Fat.	S-N-F.
	c.c.	%	%
8.2.32..	192	3·3	9·4
9.2.32..	180	1·6	9·4
10.2.32..	165	1·1	9·3
11.2.32..	158	1·5	9·4
12.2.32..	142	1·1	9·8
13.2.32..	193	1·7	9·4
16.2.32..	168	2·2	9·1
17.2.32..	189	2·2	9·8
18.2.32..	167	2·3	9·2
19.2.32..	140	1·9	9·3
20.2.32..	185	1·1	9·0
23.2.32..	180	2·0	8·5
24.2.32..	158	1·6	9·1
25.2.32..	176	2·3	8·9
29.2.32..	159	1·7	8·5
1.3.32..	164	3·3	9·6
2.3.32..	192	1·6	9·2
3.3.32..	156	2·8	8·7
4.3.32..	177	3·6	8·7
5.3.32..	156	2·4	9·5
6.3.32..	190	1·7	10·4
7.3.32..	159	2·3	9·1
8.3.32..	163	1·7	9·5
9.3.32..	118	2·7	9·6
10.3.32..	146	2·7	10·0
8.6.32..	185	3·0	9·7
Average.	112	2·3	9·3

The colostral milk period did not vary in duration from that of European breeds, the milk being fit for consumption after seven days. A fact very clearly illustrated in the table is the low butterfat percentage in numbers 3584 and 3586 (the latter was tested in two different lactation periods), as compared with the comparatively high butterfat test in the case of No. 3587. This phenomenon corroborates the findings of Blackham (1922), who states that the cow's milk in the tropics shows a fat percentage ranging from 3·4 to 7·71 per cent., a fact easily comprehended when we consider that no selection has ever been made with regard to the improvement of milk qualities.

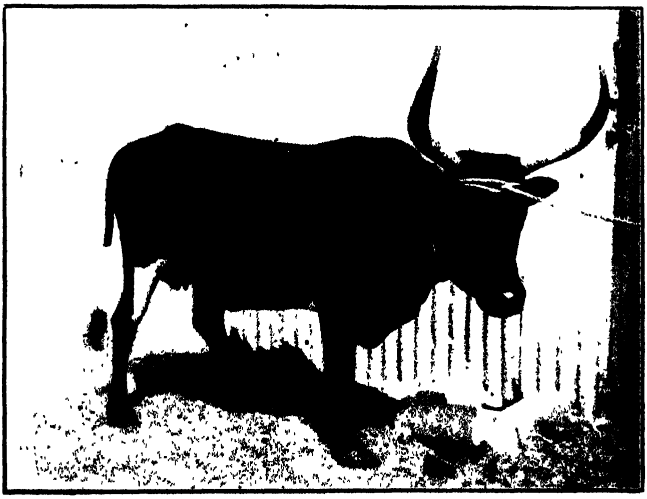


Fig. 7. Ovambo cow No. 3587.

The quantity of milk is likewise very small, but the average duration of lactation does not fall far short of European breeds. The solids-not-fat percentage is very good, comparing very favourably with the highest milk qualities.

Selection is a measure which should obviously be encouraged even among native cattle owners.

THE MEAT FROM TWO OVAMBO COWS.

Two cows, Nos. 3584 and 3588, were slaughtered. One, No. 3588, was emaciated (due to chronic metritis) and in poor compound condition, whereas No. 3584 was of medium to good condition. The carcases were very small, but quite compact, showing good width in proportion to length. The loins were rather thin and hollow, and the forequarters well fleshed, but lacking depth. There was an almost entire absence of external fat, the most noticeable layer being beneath the hump. The hump consisted chiefly of muscular tissue, and the measurements were approximately 30 cm. (length) by 20 cm. (width) and 10 cm. (depth), and the weight was about 3 Kgm.

The entire absence of marbling may be attributed to the age of the animals. The meat was of fine texture and the bones were also fine.

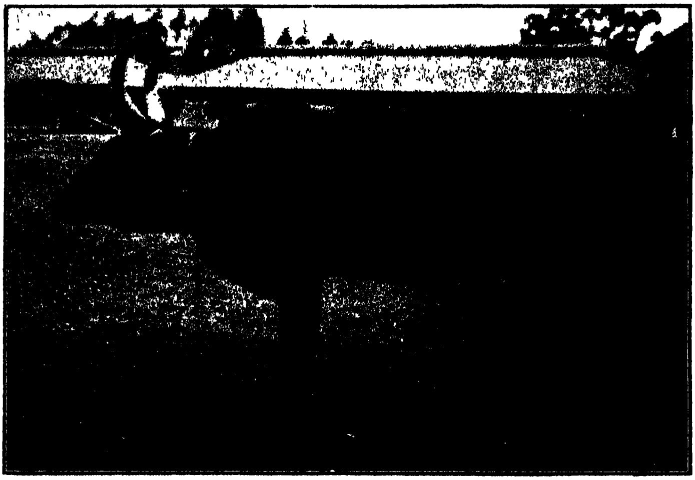


Fig. 8. Ovambo cow No. 3589.

POSSIBILITIES.

Although many breeds of *Bos taurus* are noted for their beef or milk qualties, comparatively little is known of the characteristics of the many types of *Bos indicus*, under which is grouped the Ovambo beast. In very few tropical or sub-tropical territories have the colonists or State officials endeavoured to make use of the advantages of indigenous cattle. Instead, cattle have been introduced from Europe, with the result that not only has mortality been considerable, but also a definite tendency to " degeneration' " has been noted. A little thought should convince those interested that obviously the best material for development would be the " survival of the fittest ", in other words, the stock which has survived " the vicissitudes associated with climatic, physiographic, edaphic and biotic factors."

The conditions with which the Ovambo has had to cope has given the type a fast gait so as to enable it to cover long distances during a day's grazing. In spite of this the conformation is neat and well proportioned and the type uniform. The Ovambo is thus able to thrive on poor pastures which are green only 4-5 months of the year. In addition, it possesses the advantages noted by Kelley (1932) for the Zebu, when he refers to " The indirect effect of their short sleek coat, their better provision for radiation of body heat, their smaller stomachs, different grazing methods and their ability to remain relatively long without water." It was quite evident that the Onderstepoort Ovambo cattle did not pick up ticks as readily as the long-coated grades.

The success obtained by ranchers in Texas and New Mexico by the introduction of Zebu blood is of paramount importance to us in South Africa. Apart from other advantages, e.g. disease resistance, the Smithfield market reports show that cattle of the Zebu type are worthy of attention.

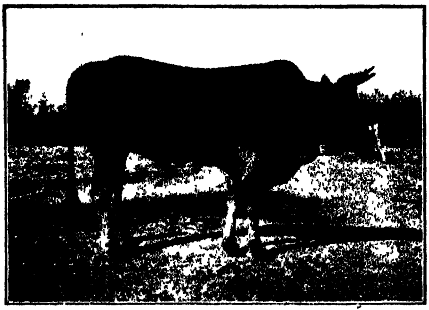


Fig. 9. Ovambo bull No. 3666.

It is clear that many decades will pass before native cattle under present management can attain the standard of excellence so desired by Europeans. The native idea of selection, e.g. pugnacity in a bull or mere coloration in a cow, etc., being so unscientific, it behoves the administrative authorities in the various tropical and sub-tropical territories to give scientific advice wherever possible. In fact, to collect data by the establishment of a herd as indicated by Quinlan, Bisschop and Curson (1932) would be the ideal.

Fig. 10. Ovambo bull calf No. 5010. (Born 3.2.32 and photographed Sept., 1932.)

ACKNOWLEDGMENT.

We desire to record our indebtedness to Prof. A. M. Bosman for his observations (Appendix 1) and to Mr. J. H. R. Bisschop for his measurements (Appendix 2). Indeed, it is evident that the data provided by these workers represent the very foundation of this study.

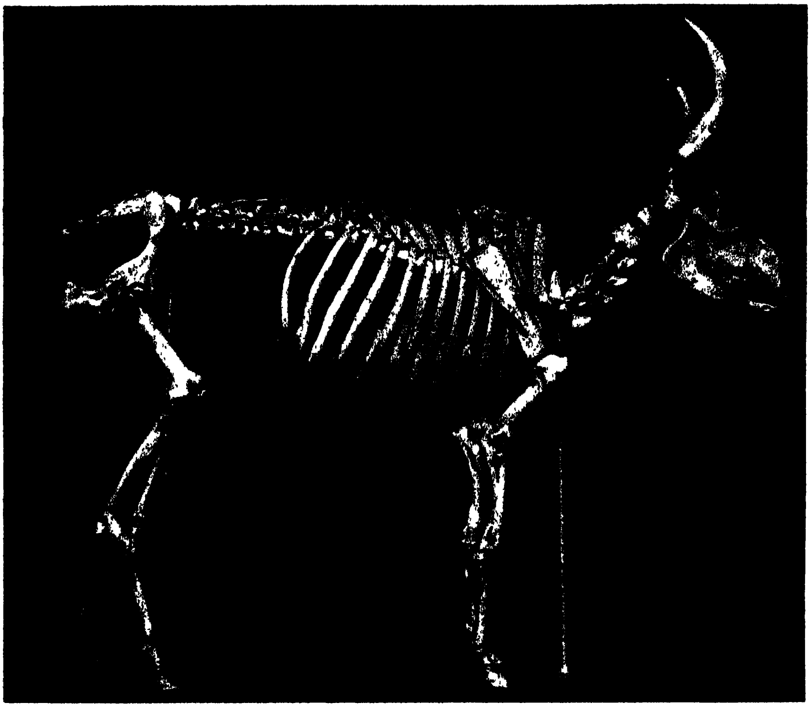


Fig. 11. Ovambo cow skeleton No. 3589. Height at withers 120 cm.

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MISCELLANEOUS.

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APPENDIX 1.

INDIVIDUAL DESCRIPTION (*See Figs.* 3-8.)

D.O.B. 3588 (taken as standard).

Head: Moderately short and fairly wide in forehead. Black strong muzzle and lips. Eye between European and Afrikander types —no large lumps as seen in Afrikander. Ears pointed. Head is straight from poll to muzzle except for a Roman nose. Poll indented.

Horn: Extends in an upward and sideward direction, bending forwards and upwards. Points slightly back. The horn is moderately oval-round at base, becoming round towards the points. Colour cream with black tips.

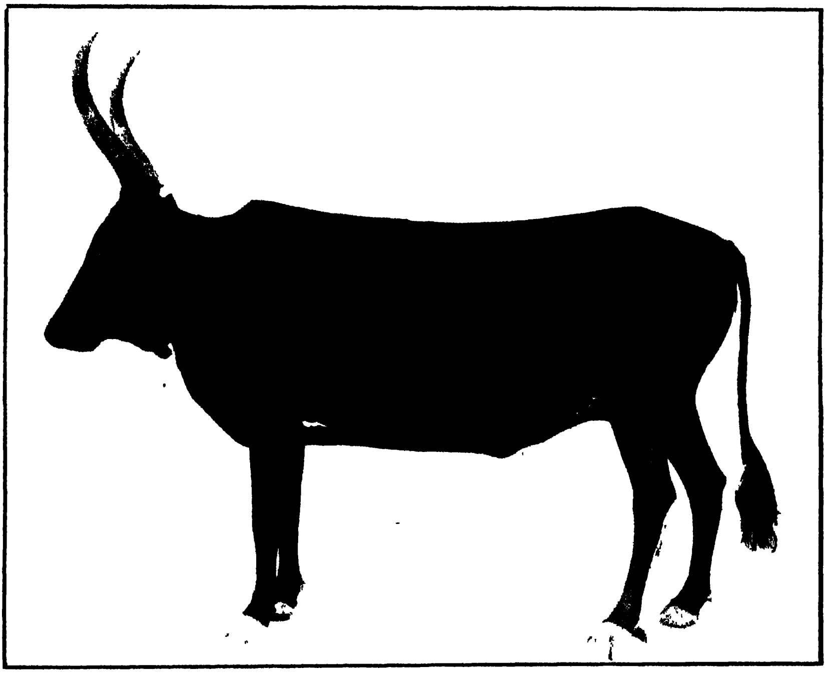

Fig. 12. Ovambo cow No. 3584, prepared for Transvaal Museum.

Neck: Fairly well developed dew-lap.

Hump: Set forward on withers and small.

Withers: Rather high and shoulder blades small and extending well up.

Chest: Moderately deep but narrow. Ribs moderately arched and depth is in proportion to the chest. Back drooping.

Navel: Smaller than in Afrikander.

Hindquarters: Very typical of the indigenous types. There is a pronounced slope towards the pin bones (*Tuber ischii*), the latter being fairly close together (pinched in). Very roofy over top of rump (roomy). Pelvic arch very pronounced.

Tail: Set high up into body. Tail itself is clean, slender, with a small switch.

Thighs and Second Thighs: Under developed.

Udder: Small but well proportioned and set fairly forward.

Legs: Clean and rather small boned. Hoofs well developed.

Colour: Dark dun with an elongated white patch on forehead and " skimmel " (white interspersed with dun hairs) mark on shoulder blade, forearm and hind flank of right side and similar marks on left side on forearm, barrel and hind flanks. Also similar marks over hook bones (*Tuber coxae*). Eyes ringed with " skimmel " marks.

Age: 11 or 12 years.

All teeth present but worn level with the gums.

General Appearance: Small and narrow with deficient hindquarters.

D.O.B. 3584 (compared with 3588).

Head: This is longer with moderately strong muzzle and lips. Horns more upstanding and longer.

Dewlap : Same.

Hump: Is slightly more developed and is nearer to the withers than in 3588.

Chest: Deep and moderately wide. Back moderately straight. Barrel well let down and ribs well sprung.

Hindquarters: Drooping, pinched in somewhat towards pin bones, fairly pronounced pelvic arch.

Tail: Moderately large and set high up. Rather short, slender with large switch.

Thighs and Second Thighs: Deficient.

Udder and Teats: Close together and rather small, rather forward.

Bone: Rather over fine, slightly cow-hocked.

Colour: Black, with " ringhals " and slight white marks on brisket and udder.

Size: Largest of the lot, and with the exception of the thighs, animal is fairly well fleshed, with long legs.

D.O.B. 3585 (compared with 3588).

Head: Same—muzzle rather small. Horns more curved inwards, with the points coming closer together.

Dewlap: Same, except that it is rather pinched round the neck (" throaty ").

Hump: Well pronounced.

Chest: Deep, but not well filled in behind the elbow. Slightly hollow back, with a well-developed and deep barrel.

Hindquarters: Short and drooping towards pin bones, which are moderately pinched in. Left hook bone more outstanding than right.

Moderately deep thigh but deficient in second thigh.

Tail: Thicker and of moderate length.

Udder: Hind teats poorly developed.

Legs: Cow hocked.

Colour: Black roan (dark grey).

Animal is rather small and is higher over hook bones than over the withers.

D.O.B. 3586 (compared with 3588).

Head: Horns similar to 3588 but thicker, larger and rounder. Very wide between horns with characteristic indented poll. Moderately short and wide head. Black skin round muzzle and eyes.

Dewlap: Fair amount.

Hump: The same.

Withers: Well rounded with fleshy shoulders.

Chest: Moderately deep and wide. Back well fleshed, slightly hollow. Ribs well sprung with a deep barrel.

Hindquarters: Moderate droop and pinched in slightly between pin bones.

Tail Head: Pronounced and set high up.

Tail: Moderately long, slender, with a good switch.

Thighs: Well developed, but lower thigh rather deficient.

Udder: Rather far forward and hind teats rather underdeveloped.

Legs: Fair bone, slightly cow hocked.

Colour: Dark dun right through, with hindquarters slightly darker than forequarters.

Size: Small, very compact, with short legs well fleshed, the animal being in good condition.

D.O.B. 3587 (compared with 3588).

Head: Differs in being weaker of muzzle and lips, shorter of horns and more hair on ear.

Dewlap: Same

Horns: Slightly oval at base and rounder towards hips.

Neck: Shorter.

Hump: Forward on withers.

Withers: Wider and more fleshy over shoulder.

Chest: Good depth and of fair width, although a little slack in the " crops ". Barrel well let down and the ribs well sprung. Back, moderately straight.

Hindquarters: Also drooping, but moderately wide between the pin bones.

Tail: Set farther back and is long and slender with a large brush.

Thighs: Lower thighs very deficient.

Udder: Placed farther back.

Legs: Shorter but otherwise similar.

Colour: Black with white markings on brisket, navel, udder, tail above switch, and in front of both thighs. In addition there is a slight " skimmel " colour round both eyes. Horns, muzzle and lips as for 3588.

APPENDIX 2.

Type : OVAMBO. MEASUREMENTS. Locality : ONDERSTEPOORT.
(Cows from Ovamboland).

D.O.B. No. and Sex.	Approximate Age. (Years).	Date of measuring (3).	Length of body (point of shoulder to *Tuber ischii*).	Height at withers.	Height at hook-bones (*Tuber coxae*).	Depth of Chest.	Width of chest (across back—behind shoulder).	Width between hook-bones.	Width between thirls (*Trochanter I, Femur*).	Width between pin-bones (*Tuber ischii*).	Heart girth.	Length of head.	Width between eyes.	Length of croup.	Greatest height. (*Tuber sacrale*).	Remarks.
Cow 3586	—	4/4/30	131·5	114·8	116·3	62	31·6	42·7	30·8	15	167·8	46	17	45·3	118·5	Condition excellent.
Cow 3587	10	„	131	108·1	112·1	60·3	39·6	44·5	32·1	13	168·3	45·5	17	43·8	115·3	Condition excellent.
Cow 3588	11–12	„	—	118·1	120·8	58·3	29	42·8	30·8	10	155	45·3	17	45·8	123·6	Condition poor.
Cow 3584	10	10/6/30	133	119	124	63	37·5	44·5	36·5	10·5	172	45·5	18	44	123	Condition good.

(3) Three measurements taken where possible and average of these is accepted. Measurements given in cm. For description of measurements see du Toit and Bisschop (1929).

APPENDIX 3.

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Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 1, Number 2, 1933.

Studies in Native Animal Husbandry: (7) *Makalanga Cattle*—A Representative Described (¹).

By J. H. R. BISSCHOP, B.Sc.Agric., B.V.Sc., and
H. H. CURSON, F.R.C.V.S., Dr.Med.Vet., Veterinary Research Officers, Onderstepoort.

INTRODUCTION.

JUST as the Pagan cattle of Nigeria represent the dwarf type of West Africa, so is the Makalanga the dwarf among South African cattle.

The most complete account of the Makalanga is that of Nobbs (1927), but as he does not give either a detailed description of the type nor illustrate his paper with photographs; these points will receive attention in this note.

Nobbs gives the limits of distribution approximately as follows: *North*, the Zambesi Valley; *east*, the Southern Rhodesia-Mozambique border; *south*, " the edge of the Limpopo Valley and to the Devuli River "; and *west*, by the Shangani River He adds that Makalanga cattle may be found beyond these boundaries. In the Union of South Africa a few specimens of the type are to be found scattered throughout the Northern Transvaal, their small size, when compared with the larger cattle of Bechuana origin, being most striking.

Nobbs correctly describes the conformation as variable, good examples being sturdy while poor specimens are undersized and weedy. Strangely enough, he makes no reference to some features characteristic of most native types, viz.: presence of small hump, well developed dewlap, and a navel fold. He mentions, however, other points, e.g. goose rump, long tail, etc.

DESCRIPTION OF MAKALANCA COW, D.O.B. 4181

(See Figs. 1 and 2.)

This cow was the calf of a Makalanga cow which was introduced in-calf from Southern Rhodesia to Northern Transvaal about 1920 by Commandant Goosen.

In May, 1930, she was purchased by Mr. S. S. Viviers from native Isak Matada on behalf of the Director of Veterinary Services and arrived at Onderstepoort on 4.6.30. The purchase price was 25s.

(¹) See *Farmer's Weekly*, 4/5/32, p. 451. Our Native Breeds.

On 13.6.30 she was described by one of us (J.H.R.B.) as follows:—

GENERAL APPEARANCE.

Diminutive in size, but in general conformation well proportioned. The most striking features were:—

- (*a*) The relatively long head, especially from the eyes downwards.
- (*b*) The relatively large and heavy horns.
- (*c*) The fineness of the bones of the limbs.
- (*d*) The presence of a small hump.
- (*e*) The low set and narrow pin bones.
- (*f*) The rootiness of the rump.

DETAILED DESCRIPTION.

1. *Head*.

Lean and dry, long and relatively narrow, especially from eyes downwards. Greatest width is found between the eyes; this width is well sustained upwards over the forehead on to a broad, slightly concave, poll. From the eyes downwards the face tapers evenly into a rather narrow muzzle. In profile the nose is uniformly convex from just below the eyes to the muzzle. The depth of head through the angle of the jaws is rather small; through the corner incisors, ditto, and reminds one of a duck's beak.

(*a*) *Forehead*: Slightly dished between eyes, sufficiently broad.

(*b*) *Eyes*: Open, of fair size. The arches, compared to the Afrikaner, are light.

(*c*) *Face*: Long and convex in profile.

(*d*) *Muzzle*: Shallow and rather narrow.

Figure 1.

Figure 2.

Fig. 1. Makalanga cow 4181. Photo by S. Viviers, May, 1930.
Fig. 2. Makalanga cow 4181.

(*e*) *Horns*: In proportion to the size of the animal, very long, large and heavy. They come away from the broad, slightly concave poll in an outward, backward and upward direction. The angle which they make with the horizontal is approximately 30°. About 10 in. from their base the horns gradually change their direction to end outwards, upwards and slightly forwards. Total length of horns about 20 in. Base, oval in cross section with an antero-posterior diameter of about 2½ in. and a latero-medial diameter of about 3½ in. Half way up, the cross section becomes circular and the horns taper gradually into sharp points. Ground colour of horns is greyish-white with dark tips.

(*f*) *Ears*: Medium in size, fine quality skin and hair, placed well below and about 4 inches behind base of horns, and carried horizontally.

(*g*) *Cheeks and Lower Jaws*: Lean and dry.

2. *Neck.*

Medium in length, good depth, flat, sufficiently covered sides, fits well on to shoulders.

(*a*) *Upper border*: Horizontal from poll to where it passes on to the hump.

(*b*) *Hump*: Small, well attached, placed well in front of withers, typically Afrikaner in type.

(*c*) *Dewlap*: On the light side, devoid of filling, starts 3 in. behind the chin as a single fold of skin, about 2 in. deep, becomes well tucked-up in the region of the throat latch and from there starts again and gradually becomes deeper to about ⅜ of the way down the neck, where it is about 6-7 in. deep. From here it again becomes smaller and ends well back between the front limbs.

3. WITHERS.

Fit in very nicely with the top line of the body; of sufficient width.

4. FRONT LIMBS.

Well placed.

(*a*) *Shoulders*: Of good depth and width, very well attached to the body and helping to form neat withers.

(*b*) *Arm*: Fair length, good musculature.

(*c*) *Fore arm*: Comparatively long, fair musculature, tapers gradually to the knee.

(*d*) *Knees*: On the small side, father round, dry and well placed between fore-arms and cannons.

(*e*) *Cannons*: Comparatively short, fine of bone, dry of tendon, of fair antero-posterior width.

(*f*) *Fetlocks*: Good size, dry and well placed.

(*g*) *Pasterns*: Straight, short, form an angle of about 50° with the horizontal.

(*h*) *Feet*: Show a toe angle of approximately 40°. Toes rather long, heels rather low, compared with the Afrikaner. Interdigital space rather wide.

5. BODY.

(*a*) *Topline*: Excellent, showing neat withers, a broad, straight, strong and well muscled back and exceptionally good loins, wide, flat, well muscled and passing over evenly into the rump.

(*b*) *Thorax*: Shows good depth of front ribs with a rather inconspicuous brisket. Hind ribs short, could be better arched and longer.

(*c*) *Abdomen*: Tucked up with cut-up hind flanks. (*N.B.*—The animal has not yet become acclimatised, feeds badly and has been scouring.)

(*d*) *Under line*: Irregular, showing a fold of skin 2 in. to 4 in. deep, running backwards from the umbilicus to the udder.

Fig. 3. Makalanga cow—a good type.

6. HINDQUARTERS.

(*a*) *Rump*: Of fair length with the sacrum running back horizontally in line with the loins, and ending in a rather heavy and high tail root. On either side of the sacrum the rump falls away badly to very low set on thirls and pin bones.

(*b*) *Hook bones*: Show good width, fairly prominent, level with the loins and the sacrum.

(*c*) *Thirls*: Rather narrow and low placed.

(*d*) *Pin bones*: Very narrow and set on low. They are quite 6 in. below the hook bones.

(*e*) *Tail*: Begins from a coarse high set on tail root, is rather heavy and ends well below the hocks in a fair switch.

7. HIND LIMBS.

(*a*) *Thighs*: From the side shows fair length dorsally, but becomes very narrow over the gaskins. From behind, the twist is badly cut up and the thighs show lack of thickness throughout. Gaskins long and narrow.

(*b*) *Hocks*: Anchylosed, due to a bilateral chronic deforming arthritis.

(*c*) *Cannons, etc.*: As in front limb.

8. MILK SYSTEM.

Udder: (Cow dry)—placed far forward on belly, shows very little udder tissue, small non-pigmented teats and practically no milk veins.

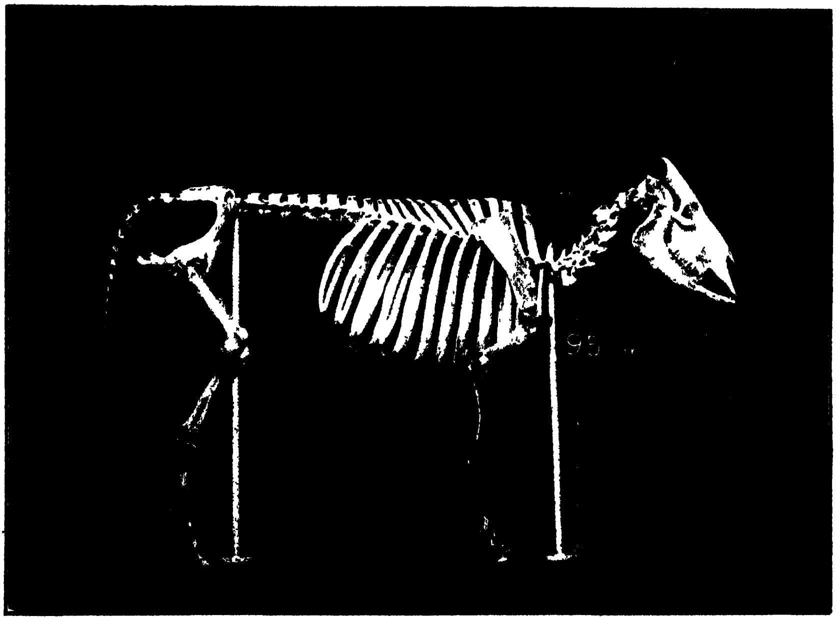

Fig. 4. Skeleton of cow 4181.

9. COLOUR.

Body colour a dark fawn, practically black; a little less dark over the upper ribs, rump and shoulders. Poll reddish brown and all along the vertebral line from the withers to the switch of the tail a red-brown band. Round the muzzie a greyish yellow band, muzzle black; dewlap, belly and switch of tail speckled grey; inner thighs yellow fawn; cannons dark fawn; hoofs black.

10. *Condition*: Poor.

11. *Weight*: 350 lb.

12. *Measurements*: See below.

TYPE: MAKALANGA.

LOCALITY: ONDERSTEPOORT.

(Cow from Northern Transvaal.)

D.O.B. No. & Sex.	Approx. Age.	Date of Measuring (2)	Length of body. (*T. ischii* point of shoulder).	Height at Withers.	Height at hookbones.	Depth of chest.	Width of chest.	Width between hookbones.	Width between thirls (*Trochanter, I. femur*).	Width between pinbones (*Tuber-ischii*).	Heart girth.	Length of head.	Width between eyes.	Length of coup.	Greatest height. (*Tuber sacrale*).	Remarks
Cow 4181	10½ years	13/6/30	108	95	102	49	28	37	27·5	9·5	132·5	42	15	36	Impossible on account of ankylosed hocks.	Lame.

(2) 3 measurements were taken and the average accepted. Measurements given in cm. For description of body measurements see Du Toit and Bisschop (1929).

CONCLUSIONS.

It must be admitted that Cow 4181 was a poor example of the Makalanga, particularly since she was affected with chronic arthritis of both hocks. A good specimen is shown in Fig. 3, this being a black cow seen on Mr. Goosen's farm near Messina, at Easter, 1930. Such conformation would be excellent as foundation stock for imported bulls, or better still, for breeding up the type.

Cow 4181 was killed on 12.9.30, her skeleton being in the Osteological Museum of this Institution (see Fig. 4).

ACKNOWLEDGMENT.

We desire to express our indebtedness to Mr. S. S. Viviers, formerly Stock Inspector at Messina, not only for Fig. 1, but also for procuring the beast described above.

REFERENCE.

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Section VI.

Miscellaneous.

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Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 1, Number 2, 1933.

Keratosis of the Skin in Cattle.

By H. H. CURSON, F.R.C.V.S., Dr.Med.Vet., Veterinary Research Officer, Onderstepoort.

MARTINAGLIA (1932) has recently drawn attention to a bovine skin affection which is to be encountered throughout Central and South Africa. In Central Africa particularly, various forms of dermatitis are exceedingly common (Curson, 1920) and offer a profitable field of study for the research veterinarian. Bee (1903) also met with the condition in question in Scotland and a photograph accompanies his note.

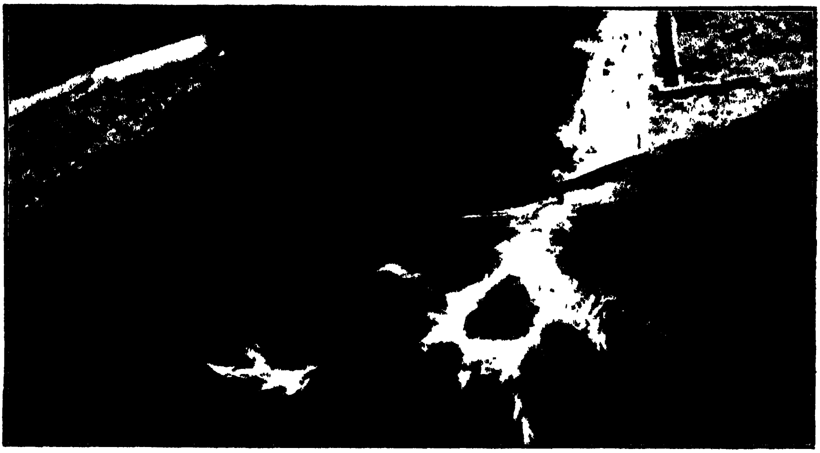

Fig. 1. Keratosis of Skin in Cattle.

"Vuursiekte", as Keratosis is sometimes called, was noticed in several head of cattle in the Eastern Cape Province in 1920, and the accompanying figure (Fig. 1) shows a lesion occurring on the right hump of a Friesland heifer. It should be added that the term "Vuursiekte" may be applied not only to any dermatitis, but also to Anthrax! As remarked by Martinaglia, the unpigmented skin is, as a rule, affected, but in the case of especially Keratosis due to branding, pigmented skin may be involved. Ox 1878 (Onderstepoort) although reddish-brown in colour, shows Keratosis, the numeral "8" in both instances being concerned.

Martinaglia also shows (Fig. 3, p. 140) a photograph of a condition encountered by the writer on several occasions in Ngamiland in 1930. It is the presence in the nasal region (just above the muzzle) of a thickening of the skin, apparently a lesion produced artificially by the natives in order to prevent weaned calves from suckling.

Further to the foregoing, an extract, referring to yet another form of bovine dermatitis, taken from the author's (Sept., 1920) monthly report from Grahamstown, C.P., is appended (Grahamstown Vet. Lab., File 76):—

" Two cases seen during the month presented the following appearances. The unpigmented skin (both animals were black and white) was bright red and painful to the touch. The coat was thin in some areas and in other places a complete loss of hair had resulted. What hair remained was dull and stood erect and obstinate ulcers had formed in the middle portion of some of the non-pigmented patches. Flakes of desquamated epidermal cells were also loosely adhering to the skin and these were easily removed by rubbing with the fingers.

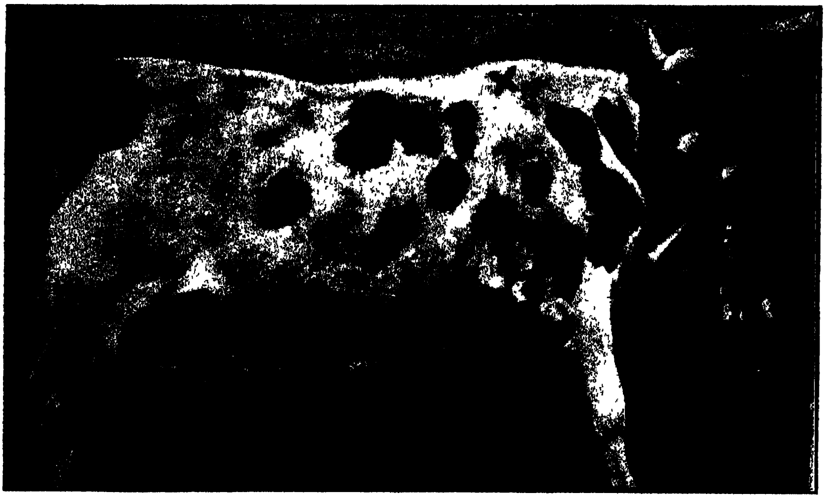

Fig. 2. Right side showing ulcer at X. The entire trunk (unpigmented) is involved.

It is important to note that there was no serious exudation such as is seen in ' Saria ' of Central Africa. Although the influence of solar rays as a possible cause must not be overlooked, yet it is interesting that the disease first appeared in mid-winter. On microscopic examination of scrapings and hairs no organisms could be detected." (See Fig. 2.)

There were, in addition, in both cases, foot lesions which took the form of a ring around each of the hoofs. The rings varied in width and depth.

REFERENCES.

BEE, J. (1903). Horny Tumour in the Skin of a Cow. *Vet. Rec.*, 3rd Jan., 1903, p. 414.

CURSON, H. H. (1920). Saria. *Vet. Jnl.*, Nov., 1920, pp. 405-412. It is convenient to mention here that this article contains three photographs. Three headings, meant to explain the photographs, were in error incorporated in the text. Two headings (referring to Figs. 1 and 2) occur on p. 409 and the third " Rinderpest Skin Lesions " on p. 410 should have been placed under Fig. 3.

MARTINAGLIA, G. (1932). Keratosis of the Skin in Cattle. *Jnl. S.A.V.M.A.*, Sept., 1932, pp. 138-141.

Onderstepoort Journal of Veterinary Science and Animal Industry, Volume I, Number 2, 1933.

Anatomical Studies, No. 38: On an Urethral Diverticulum in a Kid.

By H. H. CURSON, F.R.C.V.S., Dr.Med.Vet., Veterinary Research Officer, Onderstepoort; and

A. R. THIEL, University of Pretoria.

THE above specimen, Routine No. 6468 (Path. Book 12,448) was kindly sent by the Government Veterinary Officer, Grahamstown (Mr. R. Paine, F.R.C.V.S.) to the Director of Veterinary Services. " The goat (2 months of age) is said to have been born with this abnormality, . . . The urine collected in this dilatation, and the owner had at times to relieve the kid by pressing the accumulated contents out ". (Minute V. 6/958/31 of 3/3/32 accompanying specimen.) See Fig. 1.

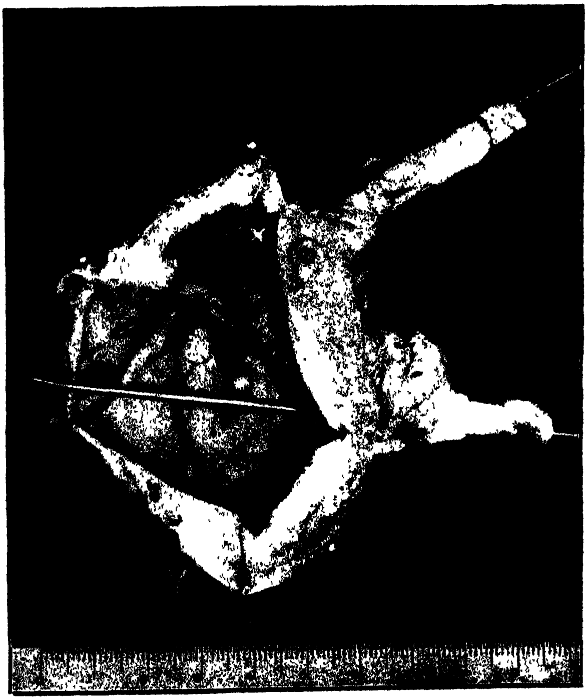

Fig. 1.

Externally all that could be seen was the pouch. On opening this, the capacity was ascertained to be approximately 30 c.c. Further, a shelf-like projection partially divided the cavity into an upper and lower portion. The former possessed a thick wall (0·75 cm.) of which the interior was rough, while the latter had a thin (0 2 cm.) smooth inner surface As will be noted in Fig. 1 it was possible to insert probes into the urethral canal both caudally towards the bladder and cranially towards the exterior, both these openings being beneath the shell-like projection.

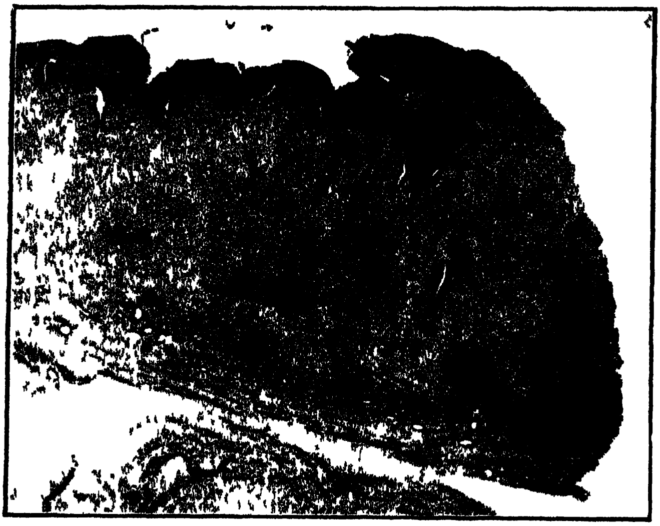

Fig. 2 (× 8).

Histologically, sections cut from the thickened wall at X, i.e. above the "shelf", showed an absence of epithelium and marked inflammatory changes. See Fig. 2. Preparations made from the thin portion of the diverticulum, e.g. at Y, showed the normal urethral epithelium. See Fig. 3.

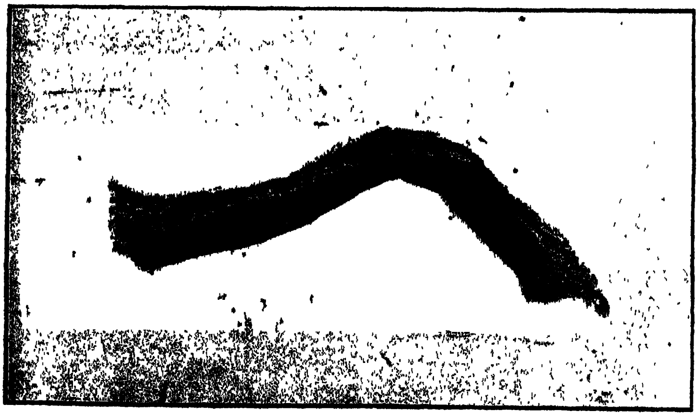

Fig. 3 (× 8).

From the above it would appear that originally there was present a diverticlum. In this, urine collected, and probably as a result of the pressure exerted by the owner (as described in paragraph 1), rupture of the sac occurred dorsally with resulting inflammatory changes. According to Hope Carlton (1932), the " explanation offered of the congenital diverticulum is that at birth there is a flap-like obstruction where that part of the urethra which arises in the cloaca joins with the part which was formed in the external genitalia. It is supposed that in a few hours increasing urinary pressure overcomes this obstruction, but there is left a tiny potential diverticulum." Later (e.g. in middle age in man) this sac may become a clinical entity.

In conclusion we desire to thank Dr. Thomas and Mr. Jackson for their assistance.

REFERENCE.

HOPE CARLTON, C. (1932). Diverticulum of the male urethra. *British Med. Jl.* No. 3712, pp. 376-377.

Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 1, Number 2, 1933.

Anatomical Studies, No. 39: A Congenital Meningeal Lipoma in a Sheep.

By H. H. CURSON, F.R.C.V.S., Dr.Med.Vet., Veterinary Research Officer, Onderstepoort.

DR. E. M. ROBINSON kindly brought me, at the end of May, 1932, the head of a cross-bred (Persian-Merino) wether, 18 months' of age, on the parietal region of which was a firm tumour (see Fig. 1). The history was that the tumour was present at birth, but that as the sheep became older, so did the " lump " become larger.

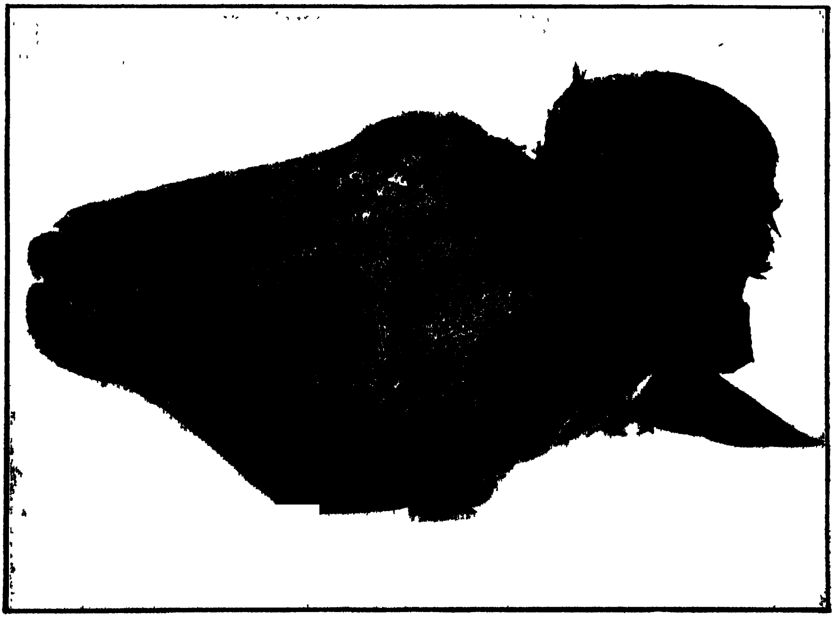

Fig. 1. A parietal tumour in a hamel.

On removal it was found to weigh 227 gm. and to have a diameter of 9 cm. and a height of 5 cm. The cranial wall, at the region of the base of the tumour, was found to be perforated by a circular opening, diameter 3·5 cm., involving the parietal and interparietal bones (see Fig. 2). Within the tumour was a cavity 2·25 cm. in width and 2·5 cm. in depth, lined presumably by the inner layer of the dura mater. Visible through the circular opening referred to above was the anterior part of the vermis of the cerebellum

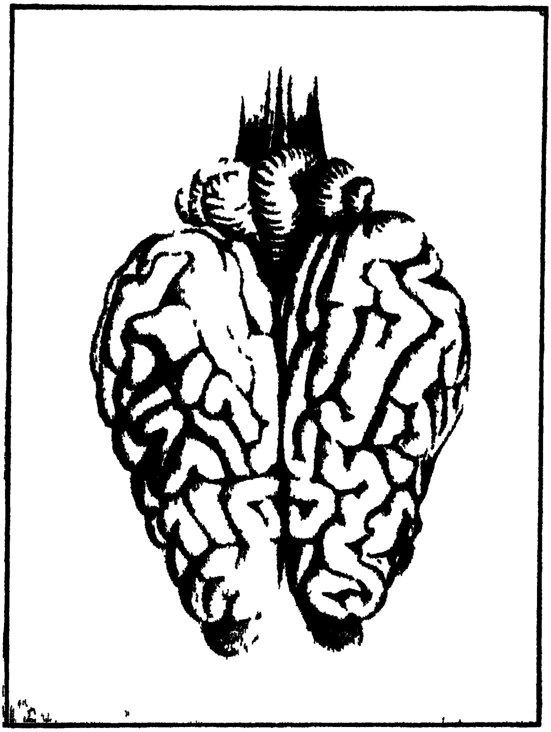

Fig. 2. (*a*) Normal.

and the posterior or occipital poles of the cerebral hemispheres. Macroscopic examination of the dorsal surface of the encephalon showed not only almost complete fusion of the cerebral hemispheres, but also an irregular arrangement of the gyri and sulci [see Fig. 2 (*b*)].

Histologically, my colleague, Mr. C. Jackson, B.V.Sc., identifies the tumour as a congenital meningeal lipoma.

Embryologically, it is believed that the growth commenced intracranially within the dura mater. Development occurred outwards before ossification of the parietal bones, and the undue pressure thus exerted no doubt contributed to the irregularity of the encephalon.

In conclusion, thanks are due particularly to J. Todd, Esq. P.O. Immerpan, Northern Transvaal for handing the material to Dr. Robinson.

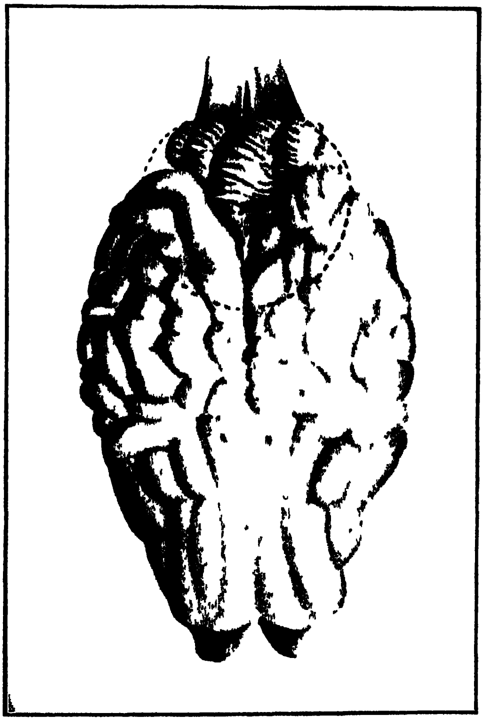

Fig. 2. (*b*) Abnormal, dotted line indicates rim of bony opening.

Onderstepoort Journal of Veterinary Science and Animal Industry, Volume I, Number 2, 1933.

Anatomical Studies, No. 40: On two Anomalies Associated with the 1st Branchial Arch.

By H. H. CURSON, F.R.C.V.S., Dr.Med.Vet., Veterinary Research Officer, Onderstepoort.

As anomalies of the 1st Branchial Arch are comparatively rare, the following cases should prove of interest:—

(*a*) UNILATERAL OTOGNATHY IN A MERINO WETHER.*
(See Figs. 1 and 2.)

The condition to be described was observed in Merino wether D.O.B. 21124 (died 12.9.1928 from phenol poisoning) and thanks are due to my former colleague, Mr. P. L. le Roux, M.R.C.V.S., for bringing me the specimen. Recently, another case (see File 258/62, Onderstepoort) came to my notice, a farmer requiring advice as to whether a ram (sire of a lamb with unilateral otognathy) was desirable for further stud purposes. The reply given was that no alarm should be occasioned by the appearance of a solitary abnormality of this nature. Dr. A. D. Thomas has seen the condition in a goat.

Although not a cyst, such a structure as indicated above, would be called a dentigerous or dermoid cyst by clinicians.

In the case in question, occurring at the anterior aspect of the base of the right *cartilago auriculae* was an accessory but miniature lower jaw. It was soft and movable and the labial portion was well defined. The anomaly measured 3 cm. long and 2 cm. at its widest part. At the back was a slitlike opening 0·75 cm. wide leading downwards and inwards to a depth of 3 cm. There were present typical papillae on the upper surface and the usual hairy covering on the lower surface. A firm wart-like structure on the upper surface resembling gum tissue contained an incisor-like tooth.

---

* Mr. C. C. Wessels, B.V.Sc., Government Veterinary Officer, Bloemfontein, informs me (30/3/33) that a full mouth Merino hamel belonging to a Mr. Victor of Brandfort, Orange Free State, shows this anomaly, the jaw in question (situated at base of left ear) actually secreting saliva. The tooth present is a well-formed incisor tooth.

As to the nature of the teratoma, such a foetal inclusion is sometimes called a parasite, true parasitism implying origin from all three germ layers. According to Bailey and Miller " it is sometimes very difficult, even impossible, to distinguish between true parasitic inclusions and dermoid cysts that are derived from ectoderm " (p. 609).

Figs. 1 and 2. Unilateral Otognathy in a Merino wether.

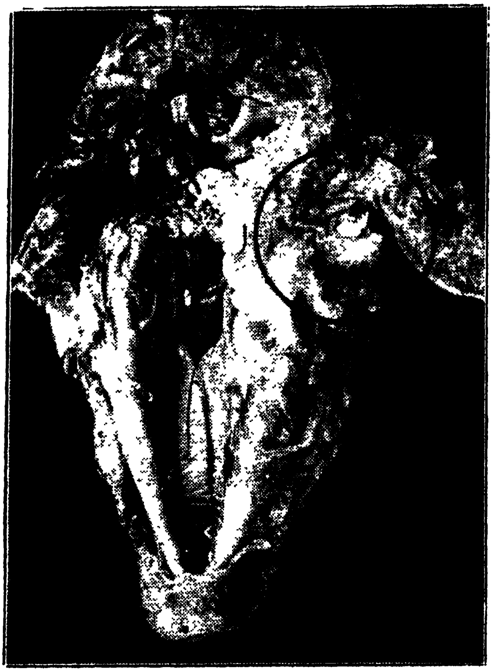

Figure 1.

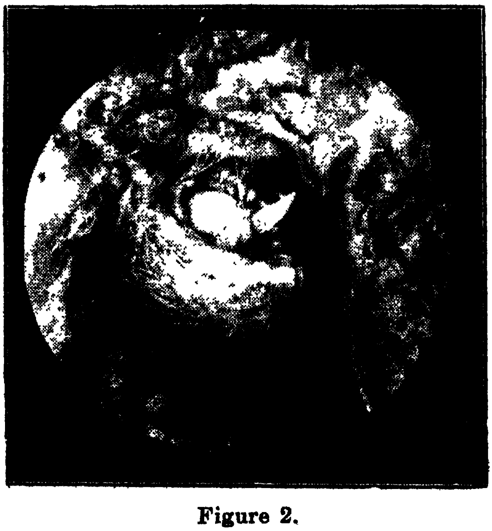

Figure 2.

Such anomalies are not infrequently found near the line of fusion of embryonic structures, e.g. region of branchial arches. In the specimen under consideration it is clear that it is derived from the 1st Branchial Arch for the ectodermal covering of the Arch is responsible for the epidermis of the lower lip and jaw and for the enamel. Both these derivatives, as can be seen in Fig. 2, are clearly defined.

As to origin, a common view is that a portion of tissue (or even blastomeres) in some way becomes detached from the parent structure and continues to grow in an abnormal situation.

Figs. 3 and 4. Inferior Dignathy in a calf.

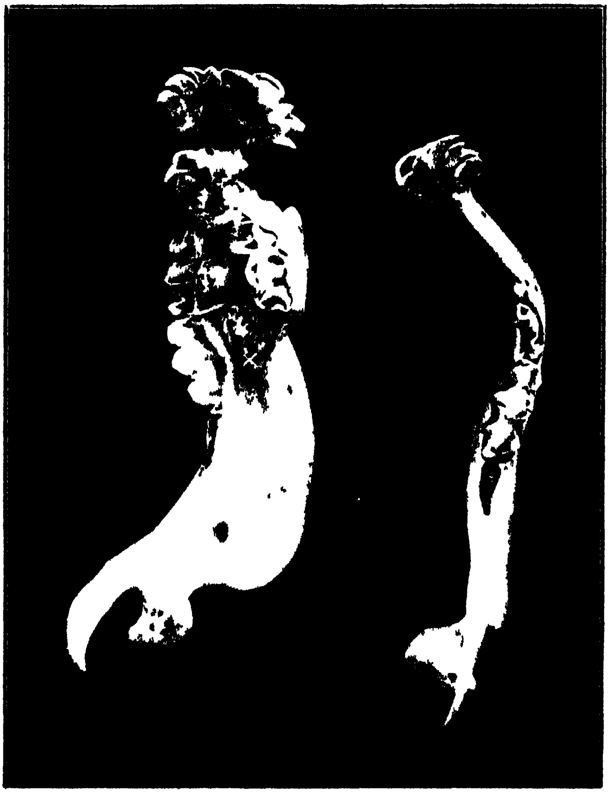

Figure 3.

It may be remarked that a common anomaly in the horse, and which is sometimes accompanied by a pre-auricular fistula (Dollar, 1912), although termed a dentigerous cyst, and associated with maldevelopment of the 1st Branchial Arch, is of a different nature. On examination a tooth or teeth of molar pattern may be found in the malar bone and to this abnormality the name *Odontoteratoma branchiale* has been given. (Kitt, 1921.)

(*b*) INFERIOR DIGNATHY IN A CALF.

(See Figs. 3 and 4.)

This specimen (T. 24—Path. No. 10783), received 9.10.1930, shows (see Fig. 3) not only an outward curving of each half of the "normal" *mandibula*, especially opposite the 2nd deciduous premolar; but also a definite twist in the region of the *corpus mandibulae* towards the junction of the right *maxilla* and *premaxilla*. The *corpus* shows altogether 7 incisors. The space thus provided accomodates an

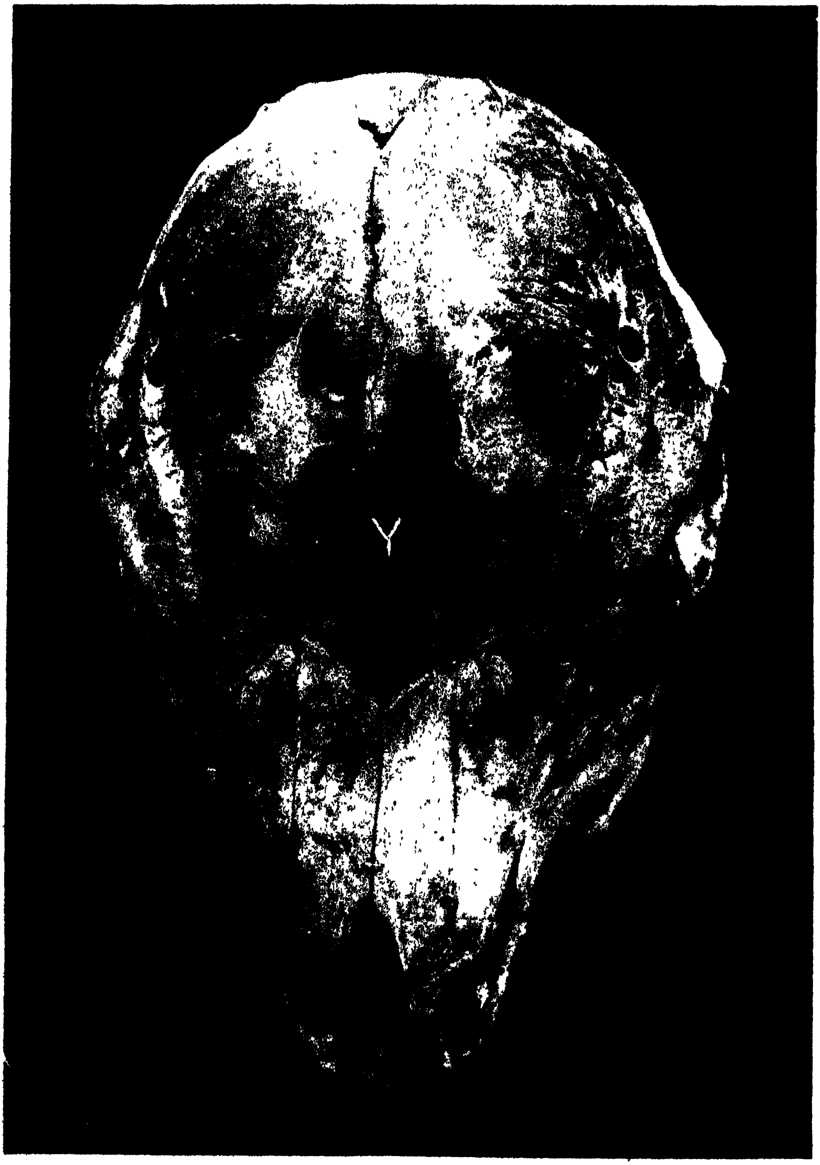

Figure 4.

ill-developed additional but miniature *mandibula*, each *pars molaris* of which shows a pair of cheek teeth. At the anterior end of the right half of the additional *mandibula* is portion of a *corpus mandibulae* containing two incisor teeth. It would appear that this was originally attached to the *corpus* of the right half of the " normal " *mandibula*, which it will be noticed is provided with four incisors. The right half has been purposely detached for photographing. Owing to the hot and dry atmosphere the teeth have in many cases become fractured.

At the point X the abnormal lower jaw was attached by a strong process to the right palatine bone of the upper jaw, which now calls for comment. As Fig. 4 indicates, there is an additional pair of nasal bones, including two nasal septa, and an additional unnamed bone situated between the frontal and nasal bones (see Y). As the specimen was received cleaned of soft tissue, the precise nature of the anomaly is not known, but it would appear that it represents a slight form of *diprosopus*. The palatine process of the maxilla is also wanting.

In this case not only was there some maldevelopment of the mandibular portion of the 1st Branchial Arch, but also of the maxillary part. Kitt states with regard to the causation of *dignathia inferior* (labial extremity) that it is " unzweifélhaft ", the result of amniotic pressure (amniotische stränge).

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